



**AGRICULTURAL RESEARCH INSTITUTE**  
**PUSA**







# **GENETICS**

**A PERIODICAL RECORD OF INVESTIGATIONS  
BEARING ON HEREDITY AND  
VARIATION**

**VOLUME 18 - 1933**

**WITH SEVEN PLATES AND ONE HUNDRED ELEVEN TEXT FIGURES**

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## CORRIGENDA

Volume 18, 1933

Page 134, Key to Charts, line 4, for "on known facts concerning individual" read "no known facts concerning individual."

Page 155, formula, line 6, for "
$$p = \sqrt{\frac{AB - 2Ab}{AB - Ab}}$$
" read "
$$p = \sqrt{\frac{AB - 2Ab}{AB + Ab}}$$
"

Page 183, line 10, for " $bb^{def}/y^{bb}/$ " read " $bb^{def}/Y^{bb}/$ ."

Page 185, line 14, for " $y\ sl^2\ bb^{def}/bb$ " read " $y\ sl^2\ bb^{def}/y\ bb$ ."

Page 185, lines 14, 15, and 16, for " $y\ sl^2\ bb^{def}/bb$ " read " $y\ sl^2\ bb^{def}/y\ bb$ ."

Page 387, Literature Cited, line 3, for "Genetics 18: 355-366" read "Genetics 18: 335-366."



## NILS HERMAN NILSSON-EHLE

(FRONTISPIECE)

NILS HERMAN NILSSON-EHLE was born in 1873 at Skurup, Sweden. He received his scholastic training at LUND, and in 1909 received the doctor's degree from that institution. In 1910 he was appointed Professor of Botany at LUND, and in 1917, Professor of Genetics. His work has covered a wide field, but his most important contributions have been in the field of cereal genetics.

During his earlier years Professor NILSSON-EHLE was interested in problems of plant variation and distribution. He was a member of a botanical expedition to Eastern Siberia in 1898, and later visited Denmark, England, France, and Germany. Plant hybrids attracted his attention at the beginning of his scientific career, and among his earlier works are descriptions of hybrids of *Salix* species and different forms of *Carex*.

In 1909 Professor NILSSON-EHLE began his important work on cereal genetics with his contribution *Kreuzungsuntersuchungen an Hafer und Weizen*. In this paper he showed that certain characters, especially grain color in wheat, may be dependent on one, two, or three factors, and that genetic ratios of 3:1, 15:1, or 63:1 may be obtained in second generation hybrids, depending on the number of genetic factors involved. This work was followed by work on inheritance of various morphological characters in cereals, the genetics of disease resistance and winter-hardiness, and a genetic analysis of various physiological characters. In recent years considerable work has been done on the genetic behavior of speltoid mutations in *Triticum*.

Professor NILSSON-EHLE has always been interested in the practical application of genetics to agriculture and has produced many new types of wheat and oats which have proved to be more disease-resistant and more productive than the older varieties. His work is an excellent example of a combination of theoretical genetics and practical plant breeding.



# GENE NUMBER, KIND, AND SIZE IN DROSOPHILA

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Received July 11, 1932

The literature on X-ray effects contains two fundamental ideas which, if combined, should offer a better understanding of the physical properties of the gene on the one hand, and of the mechanism by which X-rays affect living tissue on the other. The first of these ideas is due to CROWTHER. He interpreted the rates at which living cells are killed by X-rays as due to the destruction of a vital center within each of these cells. The second idea comes from MULLER's demonstration that X-rays impinging on chromatin will produce new gene mutations which, in turn, lead to new heritable characters. In order that the problem may be sharply defined, we shall confine the discussion to data bearing upon the kind and size of the genes present in an animal organism. The approach lies in a knowledge of the physical characteristics of X-rays in relation to their biological effects and to the rates at which these alterations are produced. This paper presents an analysis of this problem on data obtained from the treatment of *Drosophila* sperm with long wave-length X-rays of known energy. Sperm of the wild-type were chosen since they represent concentrated chromatin whose full effects under normal conditions are better understood than those of any other material.

## MATERIALS AND METHODS

The experiment utilizing the copper X-ray was performed first. Wild-type males and females taken from a stock which had been kept and studied in the laboratory for many years were mated. For the first cultures, 4 or 5 pairs were used to a bottle. This practice proved to be undesirable as the resulting progeny were crowded and somewhat reduced in size. In later work only pair mating was practiced. The male progeny, about 30 hours old, were placed in Petri dishes 1 inch in diameter, about  $\frac{1}{8}$  of an inch deep, and covered with silk bolting cloth. These dishes and their contained males were placed in the X-ray beam from 30 to 240 seconds to give the requisite X-ray dosage. (The X-ray exposures in these experiments were made in the laboratory of DR. RALPH W. G. WYCKOFF. The writers are indebted to him for the use of his apparatus, for calculation of the X-ray ionization values, and for his advice and counsel.)

The previously described gas tube (WYCKOFF and LAGSDIN 1930), equipped with targets of different metals, has been used to obtain the intense beams of X-rays needed for these experiments. These beams con-

sisted mainly of the K lines of copper and chromium, depending on the metal which was used as the target. Because of the loss of intensity consequent upon filtration and the desirability of having the data with the different radiations as comparable as possible, no attempt has been made to render the beams in any of these experiments more nearly monochromatic. Other data on filtered and unfiltered copper rays indicate, however, that only a moderate error is introduced by this slight heterogeneity.

The current through the X-ray tube during the irradiations was held constant at 4 milliamperes. The peak voltage was 34 K.V. as measured with a 12.5 cm sphere gap.

With the soft rays from copper and chromium it is necessary to make a correction for the amount absorbed in the layer of air between the surface of introduction and the volume giving the ionization current. The length of this air column was 3.2 cm. Taking the absorption coefficient (KAYE 1923) of Cu K radiation as  $\mu/P=8.43$  and of Cr K as 28.0, calculation shows that the measured ionizations at the irradiated surfaces due to the X-rays are 97 percent for Cu K and 89 percent for Cr K. Introducing this absorption correction and transferring to standard air conditions, the measured air ionizations of the beams used in the experiments are expressed as the saturation currents due to the ions produced in a cube 1 cm on its edge.

Except for irradiation, all males in any one experiment were treated alike. The control and irradiated males were mated singly to females having the composition scute, Bar, small wing, vermilion, tan ( $=s_c B s_m v t$ ) in one sex chromosome and white miniature Beadex ( $=w m B_x$ ) in the other. The scute Bar-chromosome prevents most of the crossing over and carries a recessive lethal effect. Complete counts of the progeny were made for these matings over a period of 8 days for copper and 10 days for chromium. (The breeding technique for the males irradiated with copper was somewhat different from that of the males irradiated with chromium. A copper irradiated male was bred to a single female, the pair being left in the culture bottle 8 days and then transferred to a second bottle for another period of 8 days. The average count of the progeny in the first bottle was determined and utilized as the number of surviving sperm for each dose. A study of the two bottles showed that the relative result was essentially the same but that the numbers of individuals found in the second bottle were much reduced as compared with the progeny from the first. In view of these facts, it was decided to leave the chromium irradiated pairs in the bottles for the full ten days and to omit the second bottle test. The differences in the absolute numbers of progeny for the survival curves of the copper and chromium series are accounted for on this basis.) After hatching commenced the Bar females were mated singly to  $w m B_x$  stock

males. The progeny of these matings were then examined for recessive sex-linked lethals or visible mutations which might have been produced. If mutations were discovered further breeding experiments were made to locate the position of these changes within the chromosomes. The general scheme of the matings is the familiar *CLB* type as shown in figure 1.

Four types of information are determined from these matings:

1. The first noticeable effect of X-ray treatment of any organism is the sterility of this organism when it is bred to those of normal fertility. This sterility in the case of males is attributable to some alteration pro-

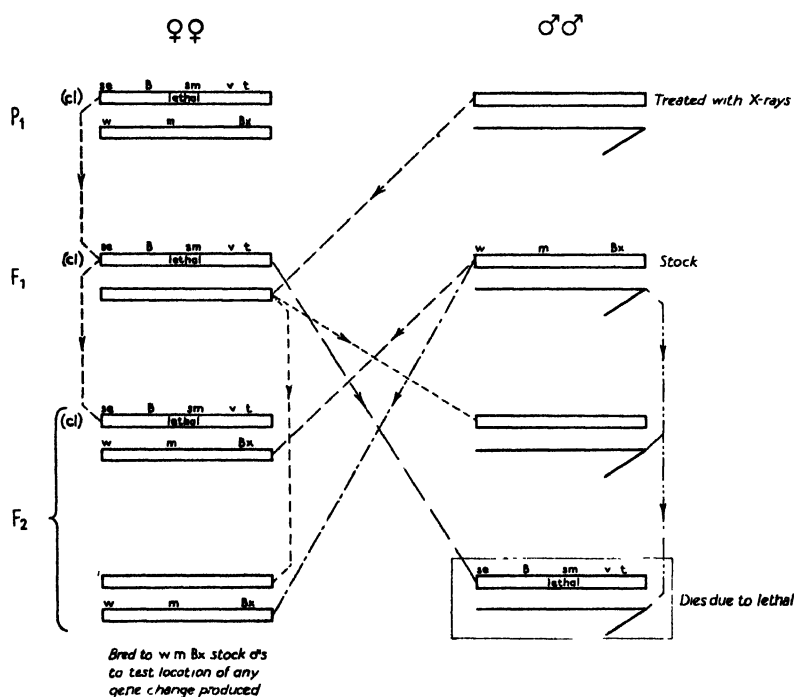


FIGURE 1.

duced within the sperm by the treatment. When such altered sperm fertilize normal eggs the embryos from these eggs die without becoming adult. The gene changes of this type produced by the X-rays may be looked upon as dominant lethals. These dominant lethals lower the number of adults surviving from matings sired by X-rayed males as contrasted with those sired by untreated males.

2. If one of these dominant lethals happens to be produced within the sex chromosome the lethal not only lowers the survival rate in this culture but it also lowers the number of females in relation to the number of males, since only females normally receive an X-rayed sex chromosome. This alteration in the sex ratio is consequently another direct measure of the number of dominant lethals produced by the X-rays.



3. The third class of data consists of the recessive lethals which are produced in the sex chromosome. These lethals manifest themselves by the fact that any  $F_1$  female carrying such a lethal will have only female progeny. As the females containing the lethal do not die they may be bred for further testing the gene mutation and for locating its position within the chromosome.

4. A fourth group of information was obtained from the numbers of matings exhibiting visible mutations. These cases were analyzed for dominant or recessive sex-linked mutants and dominant autosomal mutations.

Data were collected on these four types of information for 8 durations of X-ray and for the two classes of rays, copper and chromium.

#### METHODS OF ANALYSIS

The survival curves measuring the dominant lethal mutations produced by the X-rays for the different dosages and wave lengths are presented in table 1.

TABLE 1  
*Survival curves for Drosophila sperm tabulated against time of exposure to X-ray and total X-ray exposure.*

TIME IN SECONDS	COPPER SURVIVAL		SEX RATIO	TIME IN SECONDS	CHROMIUM SURVIVAL		SEX RATIO	C.S. U.
	NUMBER	RATIO			NUMBER	RATIO		
0	107.6 ± 10.2	1.000	1.000 ± 0.017	0	135.2 ± 11.6	1.000	1.000 ± 0.037	0
30	46.4 ± 8.7	0.431	0.960 ± 0.029	37	69.0 ± 6.7	0.511	0.965 ± 0.029	4460
60	18.9 ± 2.9	0.175	0.938 ± 0.036	72	44.7 ± 3.0	0.330	0.909 ± 0.027	8920
90	13.5 ± 2.9	0.126	0.867 ± 0.042	111	17.2 ± 1.6	0.127	0.807 ± 0.039	13380
120	7.4 ± 1.3	0.069	0.927 ± 0.052	148	5.3 ± 0.6	0.039	0.792 ± 0.064	17840
150	..	..	..	185	4.8 ± 1.1	0.036	0.934 ± 0.081	22300
180	1.4 ± 0.3	0.013	0.779 ± 0.067	222	1.3 ± 0.3	0.009	0.752 ± 0.100	26760
210	.6 ± 0.1	0.005	0.898 ± 0.108	259	0.9 ± 0.1	0.006	0.685 ± 0.090	31220
240	.4 ± 0.1	0.004	0.654 ± 0.157	..	..	..	..	35680

Ionization per sec./cm<sup>2</sup> in

1 cm of air

148.7

120.6

The survival ratios of this table when plotted on semi-logarithmic scale have certain outstanding characteristics. The raw data form a straight line of constant slope without any lag period. If we think of the X-rays as a stream of bullets shooting at random at a target, this type of curve would be generated by the condition that one hit on the vital spot of the target would kill. This vital spot may be in one piece or may be subdivided into many parts scattered throughout the target, any one of which may be hit, the total area of these smaller spots equaling the entire area of the vital spot.

Sperm may be regarded as targets composed of compact bundles of

chromatin within which the genes are embedded, one gene following another as beads do on a string. Some of these genes are vital genes in the sense that alterations in their structure produce lethals which eventually cause the death of the organism. The death resulting from such changes probably occurs in *Drosophila* in the diploid development since it was possible to show that (1) females bred to X-rayed males contained sperm in numbers comparable to those bred to untreated males, (2) hatched eggs sired by X-rayed males died at a greater rate than those sired by normal parents, and (3) sperm and eggs carrying recessive lethal genes are not perceptibly decreased in numbers.

The vital genes which X-rays change to lethal factors are distributed approximately at random within the functional chromatin as shown by these experiments. By functional chromatin is meant chromatin carrying genes, as differentiated from that of the Y chromosome and the right-hand end of the X chromosome which, up to the present, and in spite of extensive work on *Drosophila*, has not been shown to carry genes in a manner comparable with the other chromatin material.

The vital genes are of two classes, those which produce their effect in haploid condition, or to make alternative classes, dominant vital genes *versus* recessive lethal genes, and those which cause the characteristic reaction only in diploid, recessive vital genes *versus* dominant lethal genes. The deaths brought about by the X-rays as shown in table 1 may be regarded as due to the rays hitting one of these vital spots with the consequent production of dominant lethal genes.

When an X-ray strikes matter it may be absorbed or it may pass through without effect. If the ray is absorbed it gives up one or more of its quanta with the coincident production of high speed electrons. These electrons in turn give rise to secondary electrons. The volume wherein these changes take place is small. This fact, together with the random distribution of the quantum absorptions over the chromatin, presents the chance element necessary to account for the experimental results.

The mathematical analysis of the set of conditions outlined above has been presented by several investigators (CROWTHER 1926). It leads to the equation

$$\text{survival} = \frac{\text{observed survival after treatment}}{\text{initial survival without treatment}} = e^{-ant}$$

where  $a$  is the probability of one shot hitting one of these vital spots,  $n$  is the number of shots in unit time, and  $t$  is the time of exposure. The consequences of this process may be visualized from a typical calculation.

In our experiment with copper radiation 148.7 electrostatic units were given per sec./cm<sup>2</sup> in 1 cm of air.

The number of ion pairs per sec. per cm<sup>3</sup> at the position of the irradiated sperm is consequently  $148.7 \times$  electrons in one electrostatic unit or  $0.21 \times 10^{10}$  ion pairs/sec. =  $3.12 \times 10^{11}$  ion pairs. sec./cm.<sup>3</sup>

For material composed of atoms of low atomic weight these absorption coefficients vary directly with the density of the substance. If this conclusion is applied to chromatin, an assumption which cannot be far wrong,

the amount of absorption per sperm head is 
$$\frac{3.12 \times 10^{11} \times 1 \times 10^{-12} \times 1.00}{1.00 \times 1.165 \times 10^{-3}}$$

= 267 ion pairs/sec./sperm. Since direct measurement of the dimensions of the sperm head are  $7.36 \times 10^{-4}$  cm long and  $0.368 \times 10^{-4}$  cm wide, hence—considering the sperm head a rectangular block of chromatin—the volume is  $1.0 \times 10^{-12}$  cm<sup>3</sup>. The density of chromatin is assumed to be 1.00 and the density of the air at the temperature used is  $1.165 \times 10^{-3}$ .

In order to obtain the number of X-ray quanta absorbed per second it is necessary to know how many ions are liberated by one quantum absorbed in air. The best available measurements (KULENKAMPFF 1926) show that X-rays of the quality used require about 35 volts for each electron pair. The voltage equivalent of the K- $\alpha$  lines of copper follows from the quantum relation Voltage (in K.V.) =  $12.34/\text{wave length of copper X-rays}$  ( $1.537$ ) = 8.029 K.V.

The number of ion pairs arising through the absorption of one quantum

of Cu K- $\alpha$  X-rays is  $\frac{8,029}{35} = 229$ . The average number of quantum ab-

sorptions per second is  $\frac{267}{229} = 1.163$ .

The average number of absorptions which actually produced death was determined from the experimental data by fitting the curve previously derived,  $\text{survival} = e^{-\text{ant}}$ , to the actual data by the method of least squares. The resulting curve was  $Y = e^{-0.024t}$ .

If the sperm were entirely composed of vital recessive genes then every hit would be expected to kill, or the rate of killing with time should be 1.163 per second. The exponent 0.024 per second for the actual data shows that this is not the case. The chance of hitting this vital volume within the sperm clearly decreases as its volume relative to the whole sperm becomes less. The theoretical absorption 1.163 per second represents the whole volume. The effective absorption 0.024 represents the vital volume. Their ratio,  $1:0.021$ , represents the whole volume compared with this vital volume. In other words, the normal sperm area has 21 thousandths of its volume composed of vital recessive genes.

In these calculations and those which follow, extensive use is made of the data on the size of the sperm and the chromosomes composing them. These measurements were obtained from material fixed in modified Bouin and stained with iron haematoxylin. Suitable sperm which were separate and distinct from each other were photographed at 770 diameters. The photographs were then made up on lantern slides and projected on a screen. The sperm were drawn from the projected images and the magnitude of the enlargement determined. The average size of the sperm head was  $7.36 \times 10^{-4}$  cm in length and  $0.368 \times 10^{-4}$  cm in breadth. The size of the individual chromosomes within the sperm head was determined from the oögonial divisions. The technic of fixation staining and enlargement was the same as that described for the sperm save that the initial magnification of the photograph was 2500 diameters. One of the division figures utilized contained besides the normal female complex a Y chromosome. The measurements of the different chromosomes are:

Sex or X chromosome	Length $1.56 \times 10^{-4}$ cm	Average breadth $0.34 \times 10^{-4}$ cm	Area $5.27 \times 10^{-9}$ cm <sup>2</sup>
II chromosome	Length $2.21 \times 10^{-4}$ cm	Average breadth $0.33 \times 10^{-4}$ cm	Area $7.22 \times 10^{-9}$ cm <sup>2</sup>
III chromosome	Length $2.80 \times 10^{-4}$ cm	Average breadth $0.34 \times 10^{-4}$ cm	Area $9.71 \times 10^{-9}$ cm <sup>2</sup>
IV chromosome	Length $0.28 \times 10^{-4}$ cm	Average breadth $0.26 \times 10^{-4}$ cm	Area $0.58 \times 10^{-9}$ cm <sup>2</sup>
Y chromosome	Length $1.85 \times 10^{-4}$ cm	Average breadth $0.30 \times 10^{-4}$ cm	Area $5.61 \times 10^{-9}$ cm <sup>2</sup>

Total haploid size

with X chromosome Length  $6.85 \times 10^{-4}$  cm Average breadth  $0.33 \times 10^{-4}$  cm Area  $22.78 \times 10^{-9}$  cm<sup>2</sup>

Total haploid size

with Y chromosome Length  $7.14 \times 10^{-4}$  cm Average breadth  $0.32 \times 10^{-4}$  cm Area  $23.12 \times 10^{-9}$  cm<sup>2</sup>

Figure 2 shows a sample photograph of the sperm used in obtaining these measurements. Figure 3 shows one of the metaphase oögonial figures, containing a Y chromosome measured for the size of its chromosomes.

While somewhat aside from the problem under discussion, the comparison of the measurements of the sperm head with those of the condensed metaphase chromosomes is significant from the viewpoint of the mechanism by which these chromosomes may be condensed into the sperm head. The maximum breadth of the sperm head represents the maximum breadth which a chromosome assumes if the sperm is to be one chromosome wide. This breadth is  $0.368 \times 10^{-4}$  cm or somewhat smaller. The breadth of each of the chromosomes is approximately the same as this figure,  $0.33 \times 10^{-4}$  cm. If chromosomes reach the stage of extreme condensation at the metaphase, then these results show that they must lie end to end in the sperm head. The total length of the chromosomes under these conditions should be the same as the length of the chromatin in the sperm head. The length of the sperm head is  $7.36 \times 10^{-4}$  cm, although this length is again probably somewhat large. The total length of the haploid chromosomes is  $6.85 \times 10^{-4}$  with the X, or  $7.14 \times 10^{-4}$  cm with the Y.

The result therefore corresponds with the view that the chromosomes lie end to end in the *Drosophila* sperm. These measurements on fixed tissue, while somewhat less than they would be if taken from living tissue are of the same order of magnitude.

The sex ratios in the second group of data were determined from the relation  $\text{♀}/2\text{♂}$  since the presence of the lethal in the *CIB* test females reduces the males to half their true number. These sex ratios were then

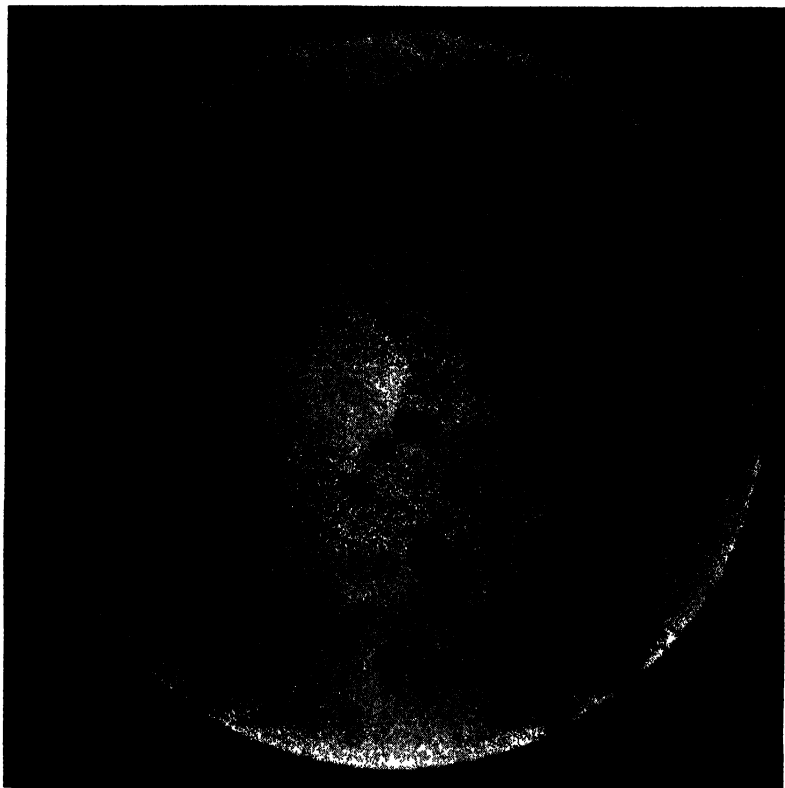


FIGURE 2.—*Drosophila* sperm taken from female. The tail of this sperm is at least three times as long as shown, and quite possibly longer since it may have been broken in handling the material. The acrosome, cytoplasmic sheath, etc. of the sperm head is stained in this preparation. Magnification 770.

adjusted to 100 as the ratio for the untreated class. The analyses of the sex ratio data were entirely similar to those for the survival curves of the whole organism as given above.

The recessive lethals could be tested and placed in their relative positions within the chromosome. The presence of one recessive lethal in general had no influence on the presence of another. It was therefore possible to determine whether or not a chromosome had more than one

lethal. This fact makes it possible to treat the data in two ways, leading to the same general conclusion.

If  $a$  is the chance of one shot hitting the target and  $n$  the number of



FIGURE 3.—*Drosophila* chromosome plate showing diploid group plus an extra Y. Magnification 2500.

shots in unit time, then the chance in a given number of units is  $Y = ant$  instead of  $e^{-ant}$  as is the case when one hit makes it impossible to distinguish another hit on the target. The rate of change is again an however.

This curve is a summation of hits, chromosomes having two recessive lethals being counted twice, those with three being counted three times, etc. Obviously this method has real difficulties when it comes to determining the presence of two or more lethals closely associated in the chromosome.

The data may also be treated exactly the same as those for the dominant lethals to obviate this difficulty. The equation for the chromosomes which show no recessive mutations, or what might be looked upon as the survivors, becomes

$$Y = \frac{\begin{array}{c} \text{observed chromosomes} \\ \text{lacking recessive lethals} \\ \text{after treatment} \end{array}}{\begin{array}{c} \text{initial chromosomes lacking} \\ \text{recessive lethals before} \\ \text{treatment} \end{array}} = e^{-ant}$$

This curve is the curve which HANSON and HEYS (1929) should have applied to their data since their technique shows only the proportion of chromosomes which had no lethal mutations and not the total number of lethal mutations per given number of chromosomes. The analysis of their beautifully regular data supports this view as the curve  $e^{-ant}$  fitted to the proportion of the chromosomes which escape being hit fits the material more accurately than the curve  $Y = ant$  worked out from the data as they present them.

#### EXPERIMENTAL DATA

Table 1 presents the data on the survival rates of sperm irradiated with increasing amounts of either copper or chromium X-ray. These data are plotted in figures 4 and 5. The middle bar for each irradiation gives the survival value attained in the experiment. The length of the heavy line shows the probable error of this determination. The data are plotted on semi-logarithmic scale since such a plot presents the curve  $Y = e^{-ant}$  as a straight line. The equations for the survival curves for the copper irradiation are  $Y = e^{-0.0244t}$  or  $Y = e^{-0.03164e \text{ s.u.}}$  depending whether time of exposure in seconds or electrostatic units of irradiation are used as the unit of measure.

The survival curves for the sperm exposed to irradiation from chromium are  $Y = e^{-0.0198t}$  or  $Y = e^{-0.03164e \text{ s.u.}}$ .

It is obvious that the curves of survival for the two treatments are the same when the electrostatic unit is used to measure the X-ray. The difference in the time curves is due to the fact that copper irradiation gives the same number of electrostatic units in less time than chromium. The straight lines of figures 4 and 5 are the theoretical curves plotted from the electrostatic unit equations.

The data on the ratios of females to males after irradiation are likewise presented in table 1. These data measure the survival value of the sex or

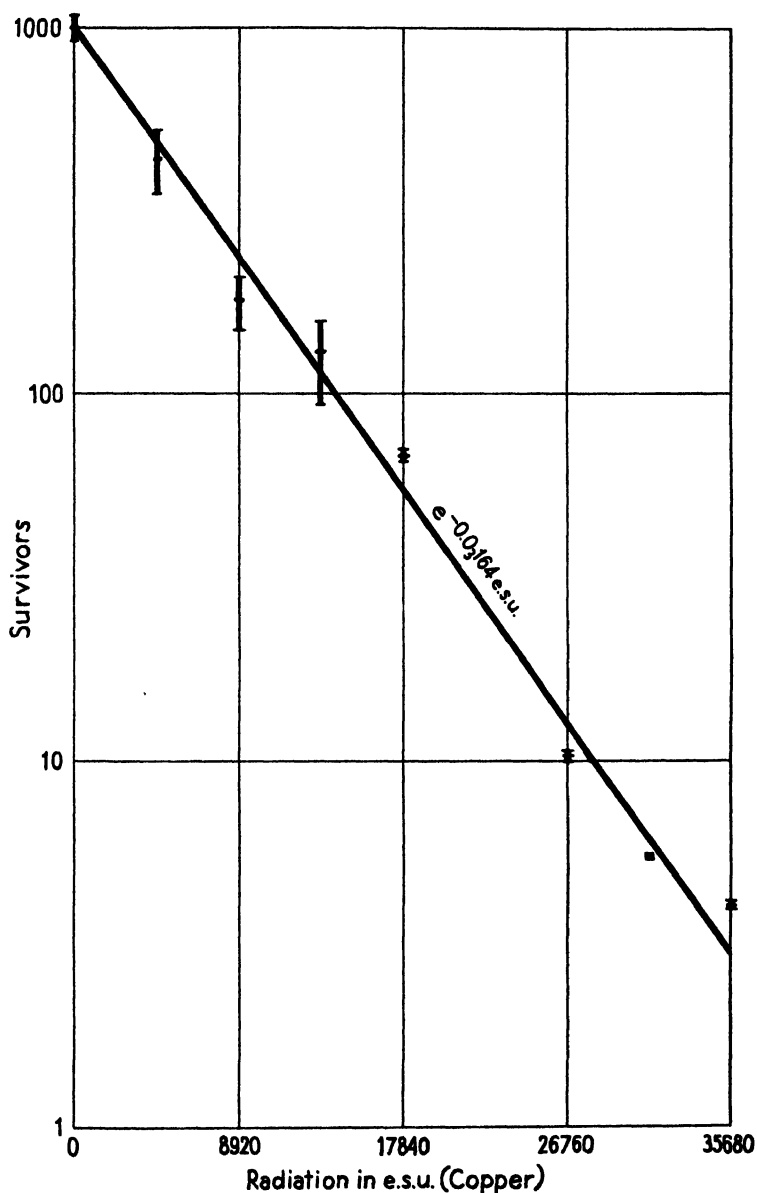


FIGURE 4.—Survival of *Drosophila* sperm irradiated with X-rays from a copper target.

X chromosome as separated from the other chromosomes, autosomes. Figures 6 and 7 present the curves for these two sex ratios plotted on semi-logarithmic scale. The cross bar shows the result of the particular treat-



ment and the heavy vertical line the probable error of this determination. There is more variation in these data than there was in those for the survival curve as the larger quantities of irradiation reduce the survivors

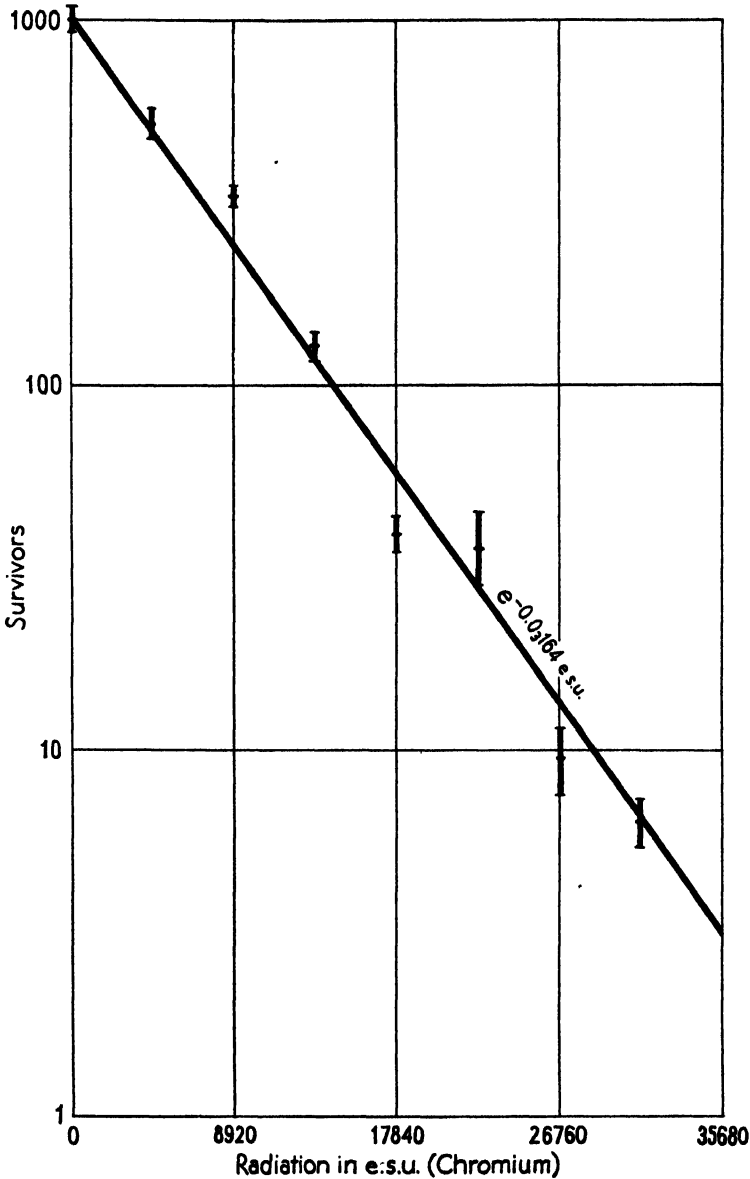


FIGURE 5.—Survival of *Drosophila* sperm irradiated with X-rays from a chromium target.

and consequently increase the observed variation. The data, however, clearly point to the linear nature of the semi-logarithmic plot. The equations for the changes of the sex ratios with increasing amounts of exposure

to the X-rays were for copper  $Y = e^{-0.00114t}$  or  $Y = e^{-0.0_3766e.s.u.}$ . The sex ratios of the flies surviving the chromium irradiation had the equations

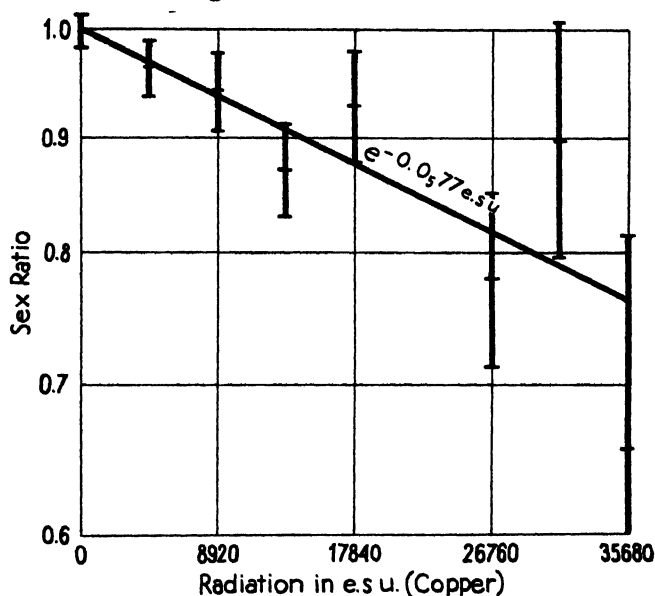


FIGURE 6.—Sex ratio of surviving *Drosophila* sperm after irradiation with X-rays from a copper target tube.

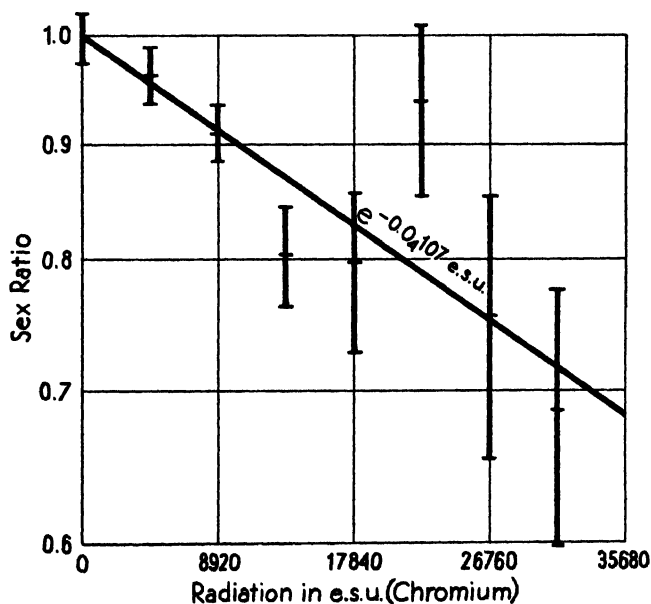


FIGURE 7.—Sex ratio of surviving *Drosophila* sperm after irradiation with X-rays from a chromium target tube.

$Y = e^{-0.00130t}$  or  $Y = e^{-0.0_4107e.s.u.}$ . The straight lines of figures 6 and 7 represent these equations.

It will be noted that the depression in sex ratio due to copper irradiation is different from that due to chromium. This difference is not significant since it has a standard error slightly more than itself ( $P = 0.35$ ).

The recessive lethal mutations furnish data which cannot be collected from the dominant lethals, as this type of mutation does not kill the fly except when homozygous. This makes it possible to separate these lethals into pure lines, locate them within the chromatin matrix, etc. These mutations appear at random in relation to each other and to the visible mutations. If they appear at random as compared with the dominant lethals then the deaths brought about by the dominant lethals do not alter the form of the curve for the rate at which the recessive lethal mutations are produced. The assumption is borne out by the data.

Were it possible to count all of the recessive lethals produced, chromosomes in which only one lethal was produced, chromosomes with two lethals, three lethals, etc., then the form of the curve, representing percentage of lethals produced against electrostatic units of irradiation to which they were exposed, would be a curve of the form  $Y = ant$ .

There is a grave technical difficulty in determining the presence of double lethals in a chromosome when these lethals are close together. It is possible however to arrive at these same constants from the curve showing the percentage of chromosomes in which no lethal is produced. This curve is entirely like the survivor curve of the dominant lethals, the ant having the same meaning as in the curve above  $Y = e^{-ant}$ .

Table 2 gives the data for the percentage of sex-linked recessive lethals, including the doubles, as they were experimentally obtained. Beside this column is given the percentage of chromosomes tested which contained no recessive lethals. The percentages of visible sex-linked factors and dominant autosomal factors are given in columns 4, 5, and 6 for the copper irradiations and 10, 11, and 12 for the chromium irradiations.

The data of table 2 lead to the equations  $Y = e^{-0.02165t}$  or  $Y = e^{-0.04112e.s.u.}$  to describe the rate of recessive lethal production for sperm exposed to copper irradiation. The rates of lethal mutation for the sperm exposed to irradiation from chromium have the equations  $Y = e^{-0.02162t}$  or  $Y = e^{-0.04135e.s.u.}$  These data are plotted in figures 8 and 9.

A comparison of figures 8 and 9 shows that the rate of lethal mutation for sperm exposed to copper irradiation is somewhat less than that from chromium irradiation. The difference in the two rates is not significant, however, since the standard error of the difference of the two rates is 0.064 and the probability that the two distributions are alike is 0.15. The curves for the percentage of mutations with time of irradiation by X-rays showed that these gene changes are produced at a constant rate irrespective of wave lengths. If percentage of mutation is plotted against energy

TABLE 2

*Relation between the X-ray dose and the ratio of recessive lethal or visible mutations.*

COPPER						
TIME IN SECONDS	SEX-LINKED MUTATIONS		RECESSIVE VISIBLE	AUTOSOMAL DOMINANTS		
	RECESSIVE LETHALS	CHROMOSOMES NOT CONTAINING RECESSIVE LETHALS		TESTED	UNTESTED	
0	0.000±	1.000±	0.000	0.0000	0.0008	
30	0.043±0.006	0.960±0.005	0.0054	0.0024	0.0062	
60	0.089±0.013	0.911±0.013	0.0097	0.0008	0.0188	
90	0.117±0.015	0.888±0.016	0.0152	0.0120	0.0142	
120	0.200±0.021	0.813±0.021	0.0375	0.0066	0.0198	
150	..	..	..	..	..	
180	0.292±0.038	0.708±0.038	0.0152	0.0234	0.0132	
210	0.350±0.051	0.675±0.050	..	0.0158	0.0158	
240	No progeny	..	..	0.0303	..	

CHROMIUM						
TIME IN SECONDS	SEX-LINKED MUTATIONS		RECESSIVE VISIBLE	AUTOSOMAL DOMINANTS		e.s.u.
	RECESSIVE LETHALS	CHROMOSOMES NOT CONTAINING RECESSIVE LETHALS		TESTED	UNTESTED	
0	0.006±0.004	0.994±0.003	0.000	0.0000	0.0030	0
37	0.068±0.010	0.935±0.010	0.0036	0.0025	0.0045	4460
72	0.124±0.011	0.878±0.011	0.0113	0.0048	0.0114	8920
111	0.193±0.017	0.815±0.017	0.0164	0.0106	0.0169	13380
148	0.265±0.036	0.779±0.034	0.0147	0.0130	0.0585	17840
185	0.188±0.029	0.825±0.029	..	0.0036	0.0216	22300
222	0.318±0.067	0.682±0.090	0.0454	0.0093	0.0278	26760
259	0.348±0.067	0.652±0.067	0.0435	0.0256	0.0342	31220
						35680

of the X-rays as the other variable, the curve shows that for a given quantity of energy the gene changes produced are constant. The wave lengths used in these experiments were rather long. HANSON and HEYS have used the gamma and beta rays of radium on *Drosophila*. In their experiments the rate of mutation, as measured by recessive lethals, increased directly with the dosage. In their first series of experiments we are unable to determine these dosages in electrostatic units. However, if we assign to one of their points a like value to ours in mutation rate, and dosage, we find that the two series of observations correspond. In another series of experiments they give a dosage of 6316 e.s.u. to their flies and obtain a mutation rate of 4.7 percent. This rate is somewhat lower than that obtained in our experiments. MULLER gives his tungsten X-ray irradiations for two points in e.s.u. units together with the rates of mutations. OLIVER gives like data which were taken in the same laboratory. For the e.s.u. readings these rates of mutation are considerably larger than those of either HANSON and HEYS or the writers. The fact that the radium

radiation produces essentially the same effects as the long waves of chromium and copper certainly suggests that wave lengths within these ranges are not important to the changes which X-rays produce in tissues.

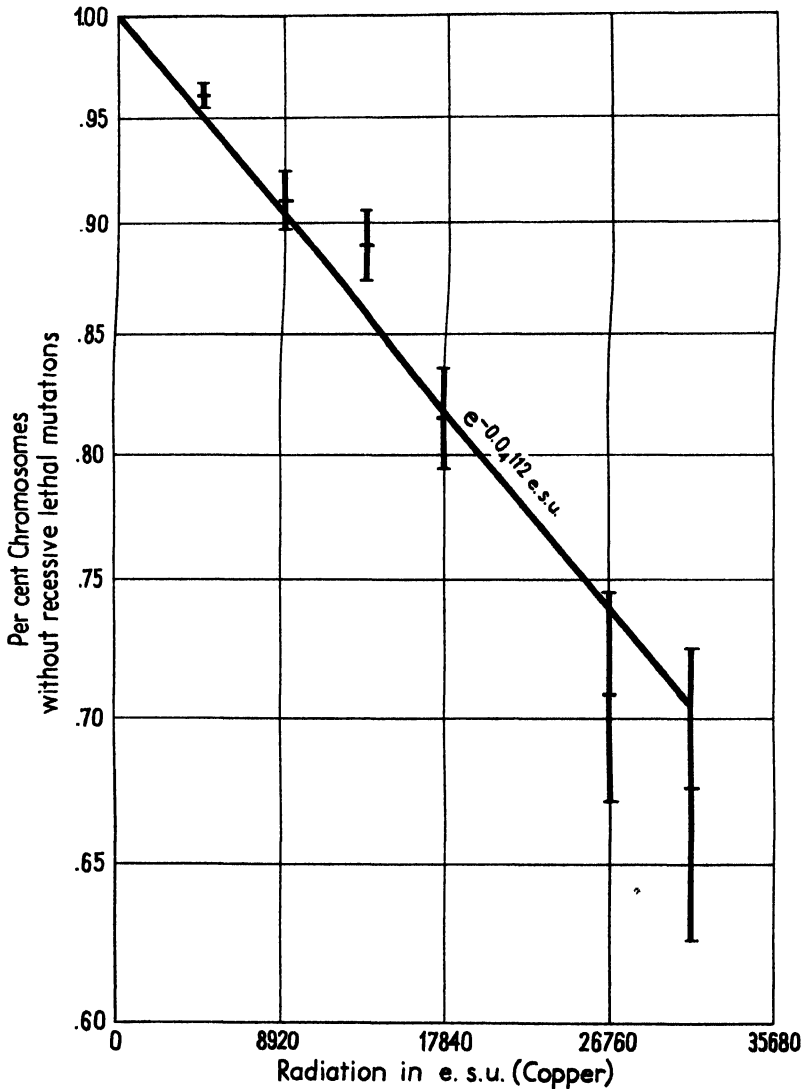


FIGURE 8.—Relation between the rate of recessive lethal mutation and exposure to X-ray irradiation from copper target.

It will be recognized that these diverse types of irradiation have produced their effects directly on the sperm without the intervention of crossing over. In this sense they would support STURTEVANT's view that crossing over must be discounted as a probable explanation of so-called natural mutation.

The visible sex-linked recessive genes which were produced by the X-ray treatments cover a rather wide category of characters. The trends of the frequency of these mutations with increasing dosages of X-ray are

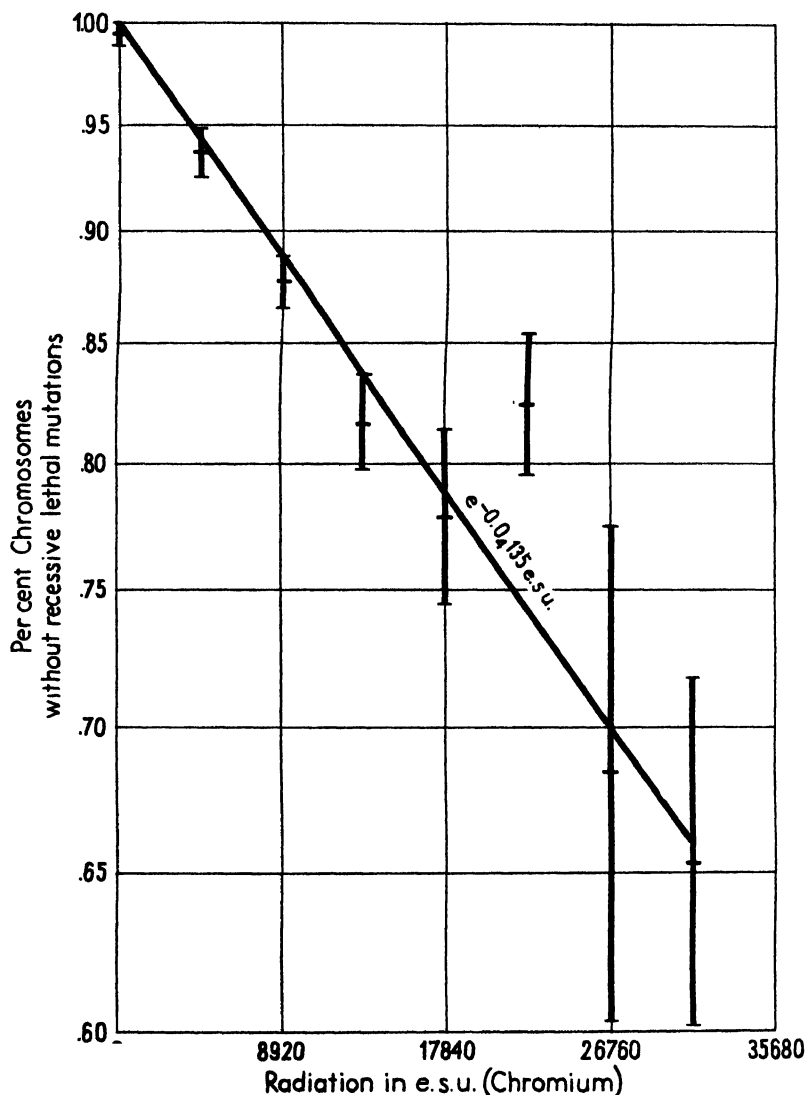


FIGURE 9.—Relation between the rate of recessive lethal mutation and exposure to X-ray irradiation from a chromium target.

given in table 2. This trend is quite irregular due to the relatively small numbers of these mutations produced. The rate of mutation rises with increase in the dosage of X-ray, however. A comparison of the rates of these mutations for the two types of X-ray leads to the conclusion that they are essentially the same. The number of visible mutations is about

one-tenth that of the recessive lethal mutations. In fact, for all data, there were 44 fairly clear and apparent visible mutations produced in the same group of sperm which produced 320 recessive sex-linked lethal mutations or 13.7 visible sex-linked mutations to 100 which were recessive sex-linked lethals.

The dominant, visible mutations in the autosomes, were divided into two groups. Each one of the first group was tested for location within its chromosome. Those of the second group failed to breed and were classified as untested. Here again it will be noted that these mutations increase with increasing X-ray dosage. The general trend of these curves is similar for both treatments; their irregularity is no doubt due to the small numbers of these mutations which were produced. The rate at which the dominant visible autosomal factors were produced is comparable with that of the recessive sex-linked visible factors. However, as the autosomes consist of about 10 times as much gene-containing chromatin as the sex chromosome, it is apparent that the dominant visible loci in the autosomes are more widely scattered than the loci for the visible sex-linked genes.

#### DISTRIBUTION OF THE GENE CHANGES

Three hundred and fifty-six mutated genes were tested for their loci within the sex chromosome. These loci were distributed over the sex chromosome's entire genetic length. The frequency distribution of the mutated genes was plotted on the conventional sex chromosome divided into units of 10, each series separately. The comparison of these distributions for the seven classes of chromosome length 0-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-70, showed a  $\chi^2$  of 10.1 for the six degrees of freedom. Because it is entirely probable ( $P=0.13$ ) that the two distributions are the same, they have been combined. Figure 10 shows these combined frequencies plotted as percent of the total mutations. The class interval for the first and second groups is 5 units of chromosome length. Each of the other intervals represents 10 units of the genetic chromosome.

Figure 10 shows much the same distribution of the mutated genes within the sex chromosome as found for the so-called natural mutation tabulated by MORGAN, BRIDGES and STURTEVANT, or for those produced by the heterogeneous irradiation of tungsten as tabulated by MULLER. (It is necessary to estimate the frequencies of MULLER's tungsten mutations from the graph, a method which, while approximately accurate, is not exact.) In common with these investigations there is an apparent close crowding of the mutation at the 0 end of the sex chromosome. This crowded area is followed by an area showing fewer gene loci. This depressed area is in turn followed by areas with mounting frequency of mutation to another crest at 50 to 60 units. From this point the number of the

genes per unit of genetic chromosome decrease to zero, at slightly beyond the 70 unit mark. One noticeable difference between our data and those cited is that no depression is noted in the region 40 to 50 units.

It seemed worth while to determine whether these three different groups of data could reasonably come from the same population. The comparison of the data of MORGAN, BRIDGES and STURTEVANT with this material showed a probability for their coming from the same population of 0.05. Comparison with the data of MULLER (1928) showed a similar probability, 0.02. The probabilities are on the borderline of significance. In the comparison over half the contribution to the  $\chi^2$  came in the first class, for on the basis of these other data, our relative frequency of factors in this

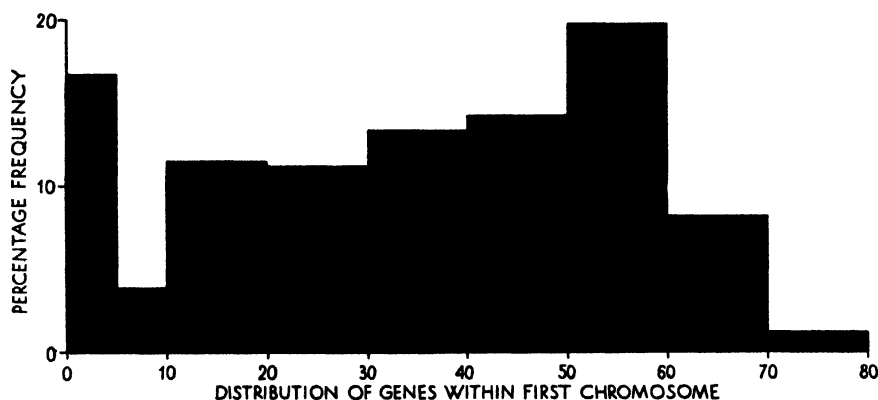


FIGURE 10.—Gene mutations as they were distributed within the genetic sex chromosome.

region is low. Another third of the contribution to  $\chi^2$  comes from the region 40–50 genetic units where we find a higher frequency of genes than do the other investigators. These differences may be significant. Since, however, the probabilities are in one case within three times the probable error and in the other but slightly beyond it, it seems likely that the differences in the three distributions are rather to be attributed to chance.

The mutant genes have been classified as lethals and visibles. These two classes intergrade and for that reason are more or less arbitrary. Figure 11 will enable the reader to appreciate the effects of these mutations on the vital processes of the organism. The data for the copper and chromium irradiations have been combined since comparison has shown them to be random samples of the same population.

Figure 11 shows that about 75 percent of this sample of genes are lethal. This means that the normal allelomorphs of 75 percent of this type of genes are vital to the animal's well-being. Seven percent show lesions which are severe enough to kill all but 1 to 5 percent of the progeny. Seven percent do not affect vital processes sufficiently to influence the organism's



viability to a marked degree. These results are comparable to those of MULLER on tungsten irradiation and of MULLER and ALTENBURG on natural mutations.

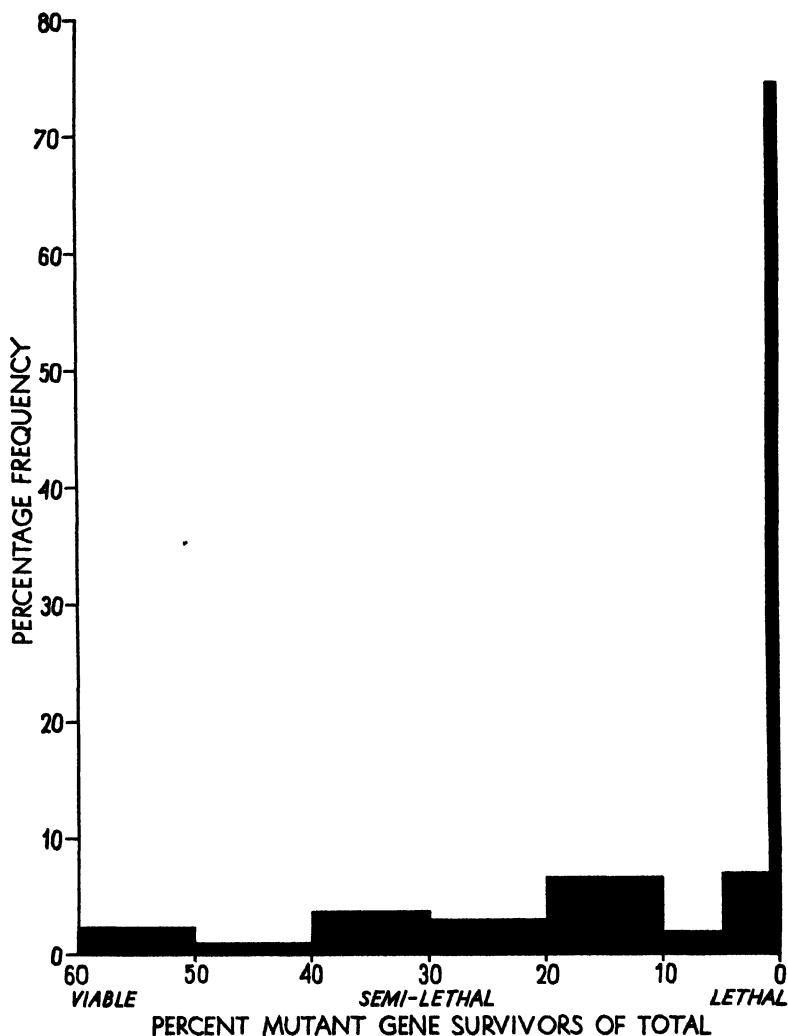


FIGURE 11.—Frequency distribution showing the sample of genes and the proportions of them which are completely lethal, produce pathogenic effects making them semi-lethal, and are fully viable. The ordinates represent percentage of genes in given class of the total. The abscissa gives the percentage of survivors in the gene mutant class divided by the whole number of survivors. 0=completely lethal; 50=normal viability.

Crossing over effects are noted with several of these factors. Aside from those which proved to be translocations between chromosomes, about 20 percent of the factors depressed the crossing over between *w* and *B<sub>x</sub>* to about half its normal value.

The exact comparison of the visible genes one with another and with those found elsewhere is of particular significance to the argument which is to follow. For this purpose we shall consider only the factors which had fair viability, defined as 20 percent or better survival, classifying the rest as lethals. With this viability it seems reasonably certain that if any one of these factors had reappeared it would have been recognized. Our experiments produced 44 such factors, and 42 factors which, although generally lethal, occasionally produced a few living mutants. (These last 42 were classified as lethals, since they might have been completely lethal had they reappeared elsewhere. In all cases these 42 mutant forms, when they did survive, showed pronounced external abnormalities, no doubt but an indication of much more extreme changes within the internal organs.)

Of the 44 gene changes, which if duplicated should be discovered, 5 were proven to be allelomorphous to genes described by MORGAN, BRIDGES and STURTEVANT. These 5 loci were yellow (some differences from the previous yellow gene), echinus twice (the second echinus also showing differences), ruby once, miniature and forked. Besides these proven allelomorphous genes there were 6 others which were probably identical with others previously described. These genes were chlorotic, facet, singed (is ♂ sterile), ascutex twice (one more extreme than the other), furrowed twice (one more extreme than the other), and fused. The criteria for the conclusion that these genes were like those described are a similar morphology and location within the chromosome.

These data allow us to form an idea of the number of these genes within the sex chromosome. As a first approximation we shall assume that all of this class of genes are equally likely to mutate under the influence of X-rays and that when they do mutate they will be equally detectable. Two samplings have been made from this total population of loci. The first sampling followed the period of years during which the evidence for the existence of any of the loci came as the chance occurrence of natural mutations, thus giving rise to a Mendelizing difference. MORGAN, BRIDGES and STURTEVANT have tabulated and described these genes. The sex chromosome genes of this series, comparable to the 44 which we have described above, fall into 42 loci. If this same population of genes is sampled, the chance of obtaining a gene occupying any one of these loci in one draw is thus  $42/\text{Total loci} \times 44$ .

The experiment showed 6 proven identical loci and 8 very probable identical loci, or 14 in all. The experimental results set the answer to the above equation as  $14; 42/\text{total loci} \times 44 = 14$  or  $\text{total loci} = 132$ . Thus the sex chromosome may contain 132 genes which can undergo rather easily detectable and fairly vital mutations. It may be argued that only the 6

proven allelomorphs should be used in the calculation. This would lead to an estimate of the number of loci as 308. We believe, however, that it is proper to consider 14 out of the 44 mutants as being allelomorphic to those previously known.

MULLER has published some data from which a similar estimate can be made. In 128 mutations of all kinds he obtained 20 which were at least 10 percent viable and conspicuous. Of these 20, 7 occupied loci previously known and tabulated among the 42 cited above; of these 7, 5 were proven allelomorphs. These data give 120 loci as the estimated number for this type of factors in the sex chromosome. Of the 7 loci found by MULLER in his tungsten X-ray work one, facet, was common to our series of 14.

The number of genes, 132, in the described category, must be regarded as a minimum, since if some of these genes mutate more readily than others we shall obtain a lower number than are actually present. Another independent estimate of the numbers of these sex-linked loci may be obtained from the ratio of the numbers of mutations noted once in the series to the numbers of those noted twice, three times, etc., after the manner developed by MULLER in his study of natural mutations. For our data 38 mutations appeared once or more; 6 appeared twice. If all of these loci are equally susceptible to the effects of the X-rays and if the genes within them change to produce equally detectable mutations then the ratio of the total number of these genes to the number that mutate once is equal to the ratio of the number that changes once to the number which changes twice, etc., or

$$\frac{N_{x+1}}{n_x} = r \text{ (a constant).}$$

The series above gives the values of  $r$  as  $6/38 = 0.158$ . The value for the total number of the gene loci in this chromosome would consequently be 38 divided by 0.16 or 238, a somewhat larger figure than that obtained by the other method.

MULLER has applied this reasoning to the data published by MORGAN, BRIDGES and STURTEVANT for natural mutations. All chromosomes were used in this determination. A very real error is introduced here since examination of the data shows that sex-linked mutations are more easily detected at their original and repeated occurrences than are the autosomal factors. Furthermore calculations from these data relate essentially to the visible factors since any arrangement of this data would make it heavily overloaded with this class. Basing the calculations on the sex-linked visible factors, we find that 7 loci mutated 8 or more times; 8 loci 7 or more; 12 loci 6 or more; 14 loci 5 or more; 15 loci 4 or more; 18 loci 3 or more; 23 loci 2 or more and 42 loci 1 or more. This series leads to the values begin-

ning with the 8 appearing loci of 0.87—0.67—0.86—0.93—0.83—0.78 and —0.55. All values save the last are remarkably alike. The last value would seem to indicate either that genes under natural conditions have idiosyncrasies which make them characteristically high, intermediate, or low in their mutation rate, or that a mutation is more apt to be described once in this summary of genes than to appear in the list of repeated appearances, a purely spurious correlation. The latter might well happen in view of the tremendous difficulties involved in accumulating such data over a period of years. However that may be, the work of STADLER would argue for the first view. The consistency of the  $r$  values after that for genes appearing only once would argue against it. If there is heterogeneity in the rates between different genes then the number of genes, 76, obtained by using the  $r=0.55$ , will certainly be too low.

MULLER has estimated the gene number by another technique. From ZELENY'S and STURTEVANT'S data on the frequency of crossing over of the Bar gene with itself he reaches a value of 0.2 of a genetic unit as the space occupied by this gene. STURTEVANT'S paper of 1925 in conjunction with his later paper of 1929 would seem to make this rate too high. If we calculate the percentages of crossing over, assigning to the data double the rate of the more viable class where necessary, and weight the 14 different types of matings by the square root of their number we arrive at the figure 0.0009 as the percentage interchange in the askew crossing over, somewhat less than a tenth of a genetic unit for the Bar gene. This reasoning would lead roughly to a total of 800 loci for the genetic sex chromosome. But as MULLER points out, there are drawbacks in this evidence; the crossover relationships are disturbed by the presence of the Bar gene, as shown by the asymmetry of crossing over, and by the fact that the Bar region of the chromosome shows a larger than normal proportion of loci when plotted against the genetic chromosome.

This discussion leads to a numerical value for the loci of sex-linked recessive genes which produce visible, easily distinguishable, characters between 75 and 268, with an average of 189. Provisionally, we shall adopt the value 175 to represent these gene loci.

The question arises as to whether the lethal genes as a class have the same mutability as the visible genes. There are several facts which suggest that they have. The apparent similarity in the distributions of these two types of genes over the chromosome as regards time and space, points to the conclusion that no one gene or even a few genes can be so susceptible to change that they account for the observed results. The conclusion that the lethal genes have the same mutability as the visible genes is borne out by another type of evidence. The reasoning may be illustrated by the chromium data where the exposure was 17800 e.s.u. The chance of obtain-

ing a recessive lethal gene change in any one sex chromosome is the number of these mutations divided by the total number of chromosomes tested, or 18 divided by 68 = 0.265. If these changes are made at random, there should be chromosomes which show two recessive lethal mutations. Such chromosomes have been found. Our technique undoubtedly does not obtain them all, however, since the difficulty of separating and proving double lethal mutations increases markedly as their loci approach each other. We shall therefore consider only those cases in which one gene occupied a position in the chromosome left of miniature and the second between miniature and Beadex. The problem may be viewed as follows. There are 36 genetic units of chromosome left of miniature and 24 units between miniature and Beadex. In our experiment 26.5 percent of mutations were obtained in 100 chromosomes. If we assume that the genetic units between the factors, yellow and miniature, and miniature and Beadex represent the proportion of potential recessive lethal factors, the ratio of the loci becomes 36 and 24. In random sampling from a bag containing balls to represent these loci there would be 36 red balls representing the loci left of miniature and 24 white balls representing the loci between miniature and Beadex. Besides these there are 167 yellow balls, a number sufficient to represent the draws when no lethal is obtained. The chance of drawing a lethal is

$$\frac{36+24}{36+24+167} = \frac{60}{227} \text{ or } 26.5 \text{ percent}$$

as in the actual experiment. In any one draw the chance of obtaining a red ball is 36/227. In the second draw the chance of obtaining a second lethal in the *mB<sub>x</sub>* region is the chance of drawing a white ball 24/227. The chance of drawing them both together is consequently 36/227 × 24/227.

But there is a second way in which this result may be accomplished since the white ball might be drawn first and the red one second. The chance of drawing a chromosome with two lethals each in the region indicated is 2(36/227 × 24/227) = 0.0335 or for the 68 draws of this experiment 2 chromosomes should be found with lethals in each of these regions. The experimental results confirm this hypothesis since we obtained 3 double lethals. Extending this process to the other irradiations the expectation of finding chromosomes with two lethals in these regions was, commencing with the initial irradiation, 4460 e.s.u., 0, 3, 3, 2, 1, 1, 1. The numbers actually obtained for the same arrangement were 1, 1, 2, 3, 1, 0, 0. The copper series is not quite in such good agreement, due partly we believe to the fact that it was the first experiment and that consequently our technique was not as good. The expectation for this series was 1, 1, 2, 3, 0, 3, 3. The observed results were 2, 0, 1, 2, 0, 0, 1. In both experiments fewer double lethals were found than expected, due partly we believe to our technique.

In spite of this difficulty the results are well within expectation since comparison of the observed with the expected leads to a  $P$  of 0.5. To this extent therefore these data support the view that recessive gene mutations are produced at random.

Testing lethal mutations directly for their rates of repeated appearance is an extremely time-consuming, tedious process, for the experiments must be performed with the autosomes. MORGAN, BRIDGES and STURTEVANT list cases of allelomorphism which have been found. The frequency of these cases is undoubtedly less than it should be when compared with the visibles which have been rediscovered, due to the technical difficulties just cited. The results so far are only useful in showing that recessive lethals do occasionally reappear.

The evidence seems to justify the assumption that the re-mutating value of the lethal factors is of the same order of magnitude as that for the visible genes. This assumption is utilized in the further discussion of the results which flow from this analysis of the experimental data.

#### DISCUSSION

The various lines of evidence may now be collected and examined in the light of the problem in hand,—the number, size and characteristics of the genes in *Drosophila*. Accepting the foregoing analysis as an essentially correct, if rough, description of the changes which take place in chromatin when *Drosophila* sperm are exposed to X-ray, and accepting the fact that there are approximately 175 visible, viable sex-linked genes, lead to the following numbers for the rest of the categories. The recessive sex-linked lethal genes are 7.3 times as numerous as the visible genes since in the experiment the ratio was 44 visible genes to 320 recessive sex-linked lethals; or there would be  $175 \times 7.3 = 1280$  loci which could be occupied by recessive sex-linked lethals. The dominant visible mutations in the sex chromosome and autosomes appeared with a frequency comparable to that of recessive visible sex-linked factors, 175. The rate at which the sex-linked recessive mutations were produced was 0.04123 for each electrostatic unit. The rate at which the dominant lethal mutations in the sex chromosome were observed was 0.0492 for each electrostatic unit. On a random chance distribution of these loci within this chromosome, the numbers of these two categories should be as their rates or 1.0:0.75. The probable number of loci which could be occupied by dominant lethals in the sex chromosome is  $1280 \times 0.75 = 960$ .

The relation of the numbers of loci in the sex chromosome to the numbers in the autosomes may be determined from the equations given earlier for the decline in the numbers which survive and from the decline in the sex ratio.

These data are gathered together in table 3. *Alpha* represents the number of quanta hitting the chromatin at a given time; *alpha* prime is the number of these quanta which are effective. As the data point to the conclusion that one quantum hit is sufficient to produce a lethal mutation, it follows that  $\alpha'/\alpha$  represents that proportion of the chromatin which is composed of these factors as related to the whole. The copper and chromium series are averaged for the volumes of chromatin occupied by the dominant lethal loci.

TABLE 3

	WHOLE SPERM CHROMATIN		SEX CHROMOSOME	
	COPPER	CHROMIUM	COPPER	CHROMIUM
Calculated $\alpha$	1.163	1.405	0.199	0.240
Observed $\alpha'$	0.024	0.020	0.0011	0.0013
Relative volume $\alpha'/\alpha$ of vital gene chromatin	Average 0.0175		Average 0.00557	

Obviously the percentage of chromatin in the whole sperm capable of producing dominant lethal mutations is considerably greater than that found in the sex chromosome. This would seem to mean that the gene containing chromatin in the sex chromosome is less than that of the autosomes since the other possible hypothesis, that the average gene size in the sex chromosome differs from that in the autosomes, seems less likely. The ratio of these two values  $0.00557/0.0175$  would consequently be the amount of gene-containing material found in the sex chromosome as compared with the autosomes, or 32.4 percent.<sup>7</sup> This figure finds important confirmation in the work of STERN, PAINTER and DOBZHANSKY. By cytological study of the size of translocations involving chromosome fragments of known genetic length, PAINTER showed that the gene-bearing chromatin of the sex chromosome was only about 1/3 the length of the whole. STERN concludes that this same length is 1/2 the chromosome. DOBZHANSKY, on the other hand, making a similar study of second and third chromosomes, reached the conclusion that while the genes are unevenly scattered as in the sex chromosome they are found along the chromosome's entire length. These data consequently furnish a direct proof of these conclusions. The effective chromatin area of the sex chromosome is 9.8 percent of that of the autosomes as derived from the relation  $5.27 \times 10^{-9} \text{ cm}^2 \times 0.325 \div 17.51 \times 10^{-9} \text{ cm}^2 = 9.8 \text{ percent}$ . From this relation the number of autosomal loci carrying genes capable of mutating into dominant lethals would be  $960/0.098$  or 9800 loci. The loci which would contain genes capable of mutating to recessive lethals would be even greater than this, 13100 loci, since the number of

<sup>7</sup> If we calculate this percentage on the chromatin rather than the whole sperm this value is 26.5. Both percentages are subject to fairly large probable errors.

dominant loci is 0.75 that of the recessive loci. The number of loci which are capable of mutating to visible recessive factors of fair viability is, on the basis of sex chromosome proportions, 1800. These estimates of loci number are listed below to facilitate comparison.

TABLE 4

TYPE OF LOCI	NUMBER	PERCENT
Sex linked loci of visible factors	175	0.6
Sex linked loci of recessive lethal factors	1280	4.7
Sex linked loci of dominant lethal factors	960	3.5
Autosomal loci of visible recessive factors	1800	6.6
Autosomal loci of recessive lethal factors	13100	48.0
Autosomal loci of dominant lethal factors	9800	36.0
All chromosome loci of dominant visible factors	175	0.6

The numbers of these loci are considerably greater than we have been accustomed to visualize. The number 1975 for the visible recessive factors is similar to MULLER's estimate except that in his estimate he considers this number as the whole number of factors,—due partly, no doubt, to the lack of information on lethals at that time. This list of factors does not include factor changes whose only expression may have been in the internal organs. This class of loci is likely to be rather small, however, since most physiological or other changes which have been noted have been accompanied by alterations in structure, which are visible externally on close examination.

The total number of loci within the *Drosophila* sperm should not be thought of as the sum of the different categories in table 4, for it seems likely that genes belonging to more than one category can occupy the same locus. We know this to be true in connection with dominant and recessive genes. It seems equally probable with regard to lethal genes since in allelo-morphic series the viability of these genes is by no means the same, some being practically lethal while others are of nearly wild-type viability. The number of loci which could be regarded as the minimum is consequently 1280 for the sex chromosome and 13,100 for the autosomes. The proportion of the various categories gives the proportion of the different types of genes which may be expected to appear under the action of an agent capable of making the genes mutate.

This tabulation demonstrates that the animal's own genetic constitution is very significant from the standpoint of pathology. It suggests that there are not less than 14,380 loci occupied by genes which are vital to the normal morphology and well-being of the organism. A single factor may cause the destruction of the legs of the fly through development of joint lesions containing melanotic pigment, or it may make the fly pin-headed in ap-



pearance and inviable, etc. Yet these visible signs are but as the red flag of the auctioneer outside the shop announcing greater things going on within. Our study of the mechanism by which the animal's own genes prevent development of pathological conditions lags far behind the studies of pathogens and the lesions they make. Yet if all the external agencies which are known to produce such changes be tabulated they fall far below the possibilities of the organism's own genes in this direction.

The estimate of the number of genes and of the volume of chromatin occupied by these genes as furnished by the X-ray results make it possible to estimate the size of an average gene. Three independent estimates of the volume of the chromatin occupied by the different classes of genes are available. The first relates to the volume occupied by the loci containing genes capable of mutation to dominant lethals; the second to like loci of the sex chromosome; and the third to loci of the sex chromosome containing genes capable of mutating to recessive lethals. The numbers of genes of each class are all estimated from the same value, 175, for the sex-linked loci of the rather viable, visible, recessive genes. These estimates for the different volumes are shown below.

	SENSITIVE VOLUME	VOLUME OF CHROMATIN OCCUPIED	VOLUME OF LOCI
Dominant lethals (sperm)	0.0175	$1.17 \times 10^{-14}$	$1.1 \times 10^{-18} \text{ cm}^3$
Dominant lethals (sex chromosome)	0.00557	$1.00 \times 10^{-16}$	$1.0 \times 10^{-18} \text{ cm}^3$
Recessive lethals (sex chromosome)	0.00752	$1.35 \times 10^{-16}$	$1.0 \times 10^{-18} \text{ cm}^3$

The sensitive volume is calculated from the methods and measurements given earlier, that is, by utilizing certain physical hypotheses. It is well to remember these hypotheses in interpreting the conclusions drawn. X-rays are assumed to be absorbed as units. An absorbed unit has a sphere of effect within which it acts. It is this purely physical quantity which is being measured. The result is biologically significant in that it sets the upper limit of the size of those elements whose alteration produces the observed results. The upper limit for the size of the gene is  $1 \times 10^{-18} \text{ cm}^3$ . This value is below microscopic vision and therefore considerably less than the chromomeres assumed by BELLING to represent genes.

The possible configuration of the space relations may be obtained from the fact that the genes within the chromosome seem to follow one another as beads on the leptotene thread. The length of the sex chromosome which carries such genes is  $0.50 \times 10^{-4} \text{ cm}$ . If we consider that every gene is capable of mutating to a recessive lethal, the total number of genes in this thread is 1280. At the leptotene stage this thread is much longer than it is in the condensed chromosome. The condensation could conceivably take place by either of two processes. The thread could contract to the length of the condensed chromosome or the thread could fold on itself in this manner

but keep its original length. If condensation by contraction took place the lineal dimension of a gene locus would be  $0.50 \times 10^{-4}$  cm  $\div$  1280 or  $3.9 \times 10^{-8}$  cm. The measurement of the sensitive volume of  $1 \times 10^{-18}$  cm<sup>3</sup> then leads to the depth and breadth dimension of  $5.0 \times 10^{-6}$  cm.

The data on organic crystals show the interatomic distance to vary between 1A and 3A. The dimension of the gene locus  $3.9 \times 10^{-8}$  cm would allow but two atoms in the lineal dimension, a value which appears too low especially when the depth and breadth dimensions are so much larger. It seems preferable therefore to regard the condensed chromosome as composed of a folded thread of leptotene dimensions where the gene locus is rectangular or spherical. Such an assumption leads to a gene  $1 \times 10^{-6}$  cm on a side or there would be 50 atoms on a side of such a figure with 125,000 in all. The average protein molecule has a molecular weight of about 120,000. The average atomic weight of such a molecule approximates 15, or there will be about 8000 atoms in such a molecule. The measurements of the gene consequently make it possible that the gene is composed of something like 15 protein molecules. The results consequently make it probable that the gene size may be reduced beyond this limit. The use of ultra-violet light for the production of mutations may offer a means to this end since the critical volume in which it is absorbed is less than that of X-rays. The difficulty which lies here, however, is that of accurately measuring the ultra-violet absorption by the sperm.

#### SUMMARY

The significant points brought out in this paper may be summarized as follows. X-rays impinging on cell chromatin cause changes at multiple foci. These changes are heritable. They fall into 3 somewhat overlapping categories, dominant lethals, recessive lethals and viable mutations producing visible somatic effects. The killing effect of X-rays on the first cell generation comes from the production of dominant lethal mutations. The death of cells of the second generation comes from the recessive sex-linked lethals produced. The rates at which the changes are produced follow specific mathematical laws so closely that they may be used to measure the X-ray dosage which is given. The rate at which these changes are produced is dependent on the ionization value in air of the delivered irradiation. The characteristic wave length of the given X-rays seems to play no part in the effects produced.

From the standpoint of those interested in pathology, the most significant findings are those showing the genetic organization of this presumably rather simple animal. The number of separate inheritable loci within such an animal is in the neighborhood of 14,380. The proportions of the genes which occupied these loci were such that

48 percent were autosomal dominant vital factors,  
 36 percent were autosomal recessive vital factors,  
 6.6 percent were autosomal recessive factors producing visible effects,  
 4.7 percent were sex-linked dominant vital factors,  
 3.5 percent were sex-linked recessive vital factors,  
 0.6 percent were sex-linked recessive visible factors,  
 0.6 percent were sex-linked and autosomal dominant visible factors.

The importance of this comparison is the fact that within any animal there are a large number of genes which have the capacity for modification and when so modified produce definite visible pathologies or death. The protective and developmental mechanism which the animal has set up through its evolution is, thus, composed of many parts. This does not seem so strange when it is realized that each and every one of these 14,380 gene loci go to every cell in the body and that were all other tissues of the body removed the body could be seen as a shadow with its present form and structure in the genes which are left.

The proof that the chromatin is not all loci-containing is a significant fact as many workers believe that the presence of thymonucleic acid is diagnostic for such material. In this light, chromatin elimination as noted in tissue has often been misinterpreted as the direct throwing off of a portion of the inheritance. The results presented show that such need not be the case. They offer an explanation for the reported observations that in bacteria and yeast chromatin like that of the higher forms is absent.

The measurement of the size of the gene seems to be the first step in the study of the organization of these genes. The data are such that we can arrive only at a maximum size. This size is  $1 \times 10^{-18}$  cm.<sup>3</sup>

Consideration of the consequences which follow from regarding the condensed chromosome as a condensed leptotene thread or as a folded leptotene thread seems to favor the fold view. Adopting this hypothesis, the gene could be composed of as many as 15 protein molecules of average atomic size.

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# THE MECHANISM OF MOSAIC FORMATION IN *DROSOPHILA*

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## INTRODUCTION

The effects of X-radiation on the chromosomes make it possible to produce several different kinds of mosaics in *Drosophila melanogaster*. In general, these mosaics fall into two classes: (1) those that result from induced point mutations, and (2) those that result from breaks in the chromosomes. It is the object of this paper to describe the mechanism underlying the formation of mosaic flies that have resulted from breaks in the X chromosome.

The method for obtaining such mosaics has been described elsewhere (PATTERSON 1931). In brief, it consists in X-raying the wild-type fly and then crossing the treated individual to one carrying an X chromosome that contains several mutant genes scattered along its length. In case the X chromosome has not been affected by the treatment the heterozygous  $F_1$  females will, of course, appear phenotypically normal. If, however, the irradiation has caused the loss, through breakage and elimination, of a piece of the treated X, the fact can be detected by the appearance of mosaic areas that show recessive characters in the  $F_1$  fly. The particular recessive characters revealed will indicate the section of the treated X that has been eliminated, because the loss of a section containing the normal or dominant genes will allow such mutant genes as lie within the corresponding section of the untreated X to be phenotypically expressed.

Not all of the variant flies arising from zygotes that have received one broken and one unbroken X chromosome will exhibit mosaicism. The appearance of mosaic tissue depends upon the condition of the chromosome at the time the break was produced. If the chromosome is broken while it is in the single strand stage, the resulting fly will not exhibit mosaic areas, but will show the deficiency in all of its tissues, constituting what we have termed an aberrant fly. Such a variant may be either a male or a female, depending upon the region of the X chromosome that has been lost. The elimination of a piece from one of the strands of the two-strand stage of this chromosome is the condition necessary for the production of mosaic tissue, for in this event only a part (usually a half) of the zygote will inherit the broken or deficient chromosome. The resultant variant will be either a sex-mosaic (gynandromorph) or a mosaic female, again depending upon the particular region eliminated.

In previous experiments dealing with this subject it was found that owing to certain difficulties inherent in the stocks ordinarily used the data obtained could not be subjected to exact statistical treatment. The chief difficulty to which I allude lies in the fact that a zygote receiving an unbroken X and another X, from which a piece had been broken off of the left end by X-rays, usually did not survive. It was found that the few aberrant females which did appear in the  $F_1$  cultures had lost but a very small piece from the extreme left end of their treated X chromosome. No female was obtained which had lost a piece by a break occurring as far to the right as the locus of prune. Since the X-radiation must break the chromosome at different levels, we should expect to find females containing chromosomes broken to the right of that locus. A study of this problem led to the discovery that the left end of the X chromosome contained a vital region, or a "gene for viability," which lies near the locus of broad. A female zygote will not develop unless this region is represented in duplicate (PATTERSON 1932a).

Attempts were made to obviate this difficulty by using as the treated parent a special stock called *Theta*. The X chromosome of the *Theta* fly carries a duplication in the form of a fragment attached to its right or fiber-bearing end. The fragment has the viability gene and the normal genes for yellow, scute, and broad, and consequently pieces of considerable length may be broken off of the left end of the *Theta* X without at the same time causing the death of a female zygote inheriting the broken element. While the *Theta* X has this great advantage over the wild-type X, yet it has one serious drawback. Aberrant females produced by breaks occurring at the left end, and falling within the range covered by the *Theta* fragment, cannot be detected in the  $F_1$  cultures, because of the dominant effect of the wild-type genes contained in this fragment.

Since the simplest and presumably the most common type of break to be expected would be one causing the elimination of the left end of the chromosome, it is especially desirable to obtain as many as possible of the variant flies that could thus be produced. The problem was then to find an X chromosome in which the viability gene lay far removed from the left end. One might expect to find such a condition in a chromosome having an inverted section. Two X chromosomes known to have inversions were tested, but in each instance the viability region was found not to be included in the inverted section. At this point Doctor S. G. LEVIT called my attention to a stock, known as scute-8 apricot, which has in its X chromosome a long inversion. This inversion extends from a point lying just to the right of the locus of scute to some point lying between the locus of bobbed and the right end of the chromosome. The genes in the inverted section occur in reverse order, and hence the locus of bobbed lies near the left end

of the reconstituted chromosome, while that of the viability gene is situated well toward the right end.

In all of the experiments given in the following section of this paper, scute-8 apricot flies were treated and crossed to untreated yellow white crossveinless miniature forked-3 flies. The diagrams for these two chromosomes are shown in figure 1. It will be clear from the diagrams that if

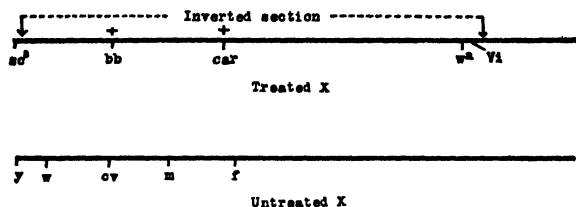


FIGURE 1.—The figure gives the diagrams of the treated and untreated X chromosomes used in the experiments. The upper diagram gives the composition of the scute-8 apricot chromosome, which contains a long inverted section. Note that the loci of white ( $w$ ) and the viability gene are located toward the right or fiber-bearing end. The lower diagram shows the mutant genes carried by the untreated chromosome. These diagrams are based on the cytological map of the X chromosome as determined by the studies of MULLER and PAINTER (1932).

the left end of the scute-8 apricot chromosome is broken off and eliminated, the female zygote inheriting the untreated X and the broken X will show yellow body color, that is, the locus of yellow will be "deficient." Such a fly will be viable because the gene for viability will not have been eliminated by the break. She will also have heterozygous apricot eyes. A similar break occurring in one of the strands of the split chromosome will produce a mosaic female that will be half yellow and half gray.

## EXPERIMENTS

### *Treated females*

In the first experiment, young females, homozygous for scute-8 and apricot, were treated with a dosage of 1325 r units and immediately mated to males belonging to the recessive stock. For the first four days the flies were transferred to fresh bottles at the end of each twenty-four hour period, and after that they were changed at the end of each forty-eight hours, until the end of the tenth day. In this, as in all of the succeeding experiments, the cultures were kept in a room in which the temperature varied from 25 to 27.5 degrees Centigrade, with an average of 26 degrees. The flies for any lot began coming out on the eighth day and all had emerged by the end of the ninth day. The  $F_1$  flies were examined for variants of all kinds, and a record kept of all individuals that had arisen from treated eggs fertilized by untreated X-bearing sperms.

The data from this experiment are given in table 1. They include for all, except the first daily period, the first five thousand flies counted for each

TABLE 1

*Scute-8 apricot females, aged 0-12 hours, treated at 1325 r units, and crossed to yellow white cross-veinless miniature forked-3 males.*

DAYS	APRICOT FEMALES	ABERRANT FLIES		SEX-MOSAICS		LETHAL MUTATIONS					TOTALS
		y w <sup>a</sup>	w c <sup>b</sup> m f <sup>c</sup>	PARTIAL	HALF	w	w-f <sup>a</sup>	f <sup>a</sup>	m	f	
		FEMALES	MALES								
1	109	0	0	0	0	0	0	0	0	0	109
2	4,983	8	2	1	3	2	0	0	1	0	5,000
3	4,988	5	0	3	0	2	0	0	2	0	5,000
4	4,994	3	0	0	1	1	0	0	1	0	5,000
5+6	4,997	1	0	0	0	1	0	1	0	0	5,000
7+8	4,999	1	0	0	0	0	0	0	0	0	5,000
9+10	4,997	0	0	3	0	0	0	0	0	0	5,000
Totals	30,067	18	2	7	4	6	0	1	4	0	30,109

period. It was not practicable to attempt to obtain this number for the first period because of the fact that young females lay very few eggs within the first twenty-four hours. The variant flies, other than those due to point mutations, are listed under the general headings of aberrants, sex-mosaics, and lethal mutations. The point mutation cases are included with the non-variant apricot females. This method of recording reveals at once the frequency of occurrence of the different types of variants produced by deficiencies, for the total number of flies listed corresponds to the number of treated X chromosomes. Furthermore, the periodic transfer method shows the time in the germ-cell cycle at which the irradiation is most effective in breaking the chromosome.

Eighteen aberrant yellow apricot females were produced in a total of 30,109 treated X chromosomes, or about one in every 1672 chromosomes. It will be noted that the greatest number occurred on the second day (practically from the first eggs laid) and that they appeared in decreasing numbers for the succeeding days. Only two aberrant males were found, and both of these appeared on the second day. Among the eleven sex-mosaics, seven belonged to the partial type, that is, the fly was practically all female, with a small amount of male tissue present, such as a sex-comb, or male genitalia, or a small area of male coloration. Such flies usually do not result from X-raying, for they occur with about equal frequency among the controls. Most of them are due to the spontaneous elimination of one of the X chromosomes at one of the late somatic divisions. Four half-and-half gynandromorphs were found, three appearing on the second day, and one on the fourth day. The male parts of the first three had lost the maternal or treated X from one daughter cell at the first somatic division, while those of the fourth case had lost the paternal X. The three that had lost the treated X were probably caused by the X-raying. The flies were examined



for variations at the marked loci for white, facet (notched flies), miniature and forked, and all cases that showed, from breeding tests, the mutation to be lethal for the male (and therefore deficient) have been included in the table. Most of these came from eggs that had been laid during the first few days.

If we consider all of the variants to which the effect of X-radiation may be assigned, their frequency for the several periods of laying are as follows: one in 312 for the second day; one in 555 for the third day; one in 1000 for the fourth day; one in 1666 for the fifth period; one in 5000 for the sixth period; and none in 5000 for the last period. From this one may conclude that the nearer the female germ cell is to maturity at the time of treatment, the greater will be the effect of the irradiation in producing breaks and other disturbances in the chromosome.

The control flies for this experiment are given in table 2. The parent flies for the control series were taken from the same lots as those for the

TABLE 2  
*Scute-8 apricot females, aged 0-12 hours, and crossed to yellow white cross-  
veinless miniature forked-3 males.*

DAYS	APRICOT FEMALES	ABERRANT FLIES		SEX-MOSAICS		LETHAL MUTATIONS					TOTALS				
		y	w <sup>a</sup>	w	c <sub>r</sub>	m	f <sub>3</sub>	PARTIAL	HALF	w		w-f <sub>a</sub>	f <sub>a</sub>	m	f
		FEMALES		MALES											
1	502	0	0	0	0	0	0	0	0	0	0	0	0	502	
2	4,997	0	0	2	1	0	0	0	0	0	0	0	0	5,000	
3	4,998	1	0	1	0	0	0	0	0	0	0	0	0	5,000	
4	4,997	0	0	3	0	0	0	0	0	0	0	0	0	5,000	
5+6	4,999	0	0	1	0	0	0	0	0	0	0	0	0	5,000	
7+8	4,999	0	0	1	0	0	0	0	0	0	0	0	0	5,000	
9+10	4,999	0	0	1	0	0	0	0	0	0	0	0	0	5,000	
Totals	30,491	1	0	9	1	0	0	0	0	0	0	0	0	30,502	

experimental series, and were handled in exactly the same manner, except that the X-ray treatment was omitted. The only variant flies found were nine partial sex-mosaics, one half and half gynandromorph in which the male parts had lost the maternal X, and a single aberrant female.

The second experiment was similar to the first. In this, however, the females were "aged" for seven days before giving the treatment and mating to the recessive males. The counted F<sub>1</sub> flies are listed in table 3. The total number of flies obtained for the seven periods was 30,506 which is an increase of almost 400 over that secured in the first experiment. The increase is confined, of course, to the first day. The older females lay many eggs on this day, although the vast majority of them fail to develop. Under these conditions the cultures tend to become sour. In a measure, this difficulty can be overcome by "seeding" the food bottles with eggs laid for a

TABLE 3

*Scute-8 apricot females, aged seven days, treated at 1325 r units, and crossed to yellow white crossveinless miniature forked-3 males.*

DAYS	APRICOT FEMALES	ABERRANT FLIES		SEX-MOSAICS		LETHAL MUTATIONS					TOTALS
		y w <sup>a</sup>	w c <sub>v</sub> m f <sub>3</sub>	PARTIAL	HALF	w	w-f <sub>a</sub>	f <sub>a</sub>	m	f	
		FEMALES	MALES								
1	504	2	0	0	0	0	0	0	0	0	506
2	4,980	14	1	1	0	-1	1	1	1	0	5,000
3	4,981	13	2	2	0	1	1	0	0	0	5,000
4	4,987	12	0	0	1	0	0	0	0	0	5,000
5+6	4,994	1	0	2	1	1	0	1	0	0	5,000
7+8	4,996	0	0	2	2	0	0	0	0	0	5,000
9+10	4,999	0	0	1	0	0	0	0	0	0	5,000
Totals	30,441	42	3	8	4	3	2	2	1	0	30,506

few hours by foreign flies, whose offspring can be easily recognized (for example, wild-type), before introducing the treated flies. The foreign larvae work the culture so that more individuals of the desired kind succeed in developing.

Among the F<sub>1</sub> flies of this series were found fifty-seven variants that could be attributed to the effects of X-rays. The frequency of their occurrence is one in every 250, 263, 294, 461, 1250, 2500, respectively, for the first six periods of laying, and one for the seventh or last period. The effect of aging virgin females, before treatment and mating, on the production of variants is clearly seen. As we have shown elsewhere, this is due to the fact that virgin females aged for several days retain most of their fully developed eggs in the ovaries, and, consequently, they have more eggs that are susceptible to the effects of radiation than is the case of younger or unaged females (PATTERSON, BREWSTER, and WINCHESTER 1932).

The control flies for the second experiment are given in table 4. Eleven

TABLE 4

*Scute-8 apricot females, aged seven days, and crossed to yellow white crossveinless miniature forked-3 males.*

DAYS	APRICOT FEMALES	ABERRANT FLIES		SEX-MOSAICS		LETHAL MUTATIONS					TOTALS
		y w <sup>a</sup>	w c <sub>v</sub> m f <sub>3</sub>	PARTIAL	HALF	w	w-f <sub>a</sub>	f <sub>a</sub>	m	f	
		FEMALES	MALES								
1	2,113	0	0	2	0	0	0	0	0	0	2,115
2	4,999	0	0	1	0	0	0	0	0	0	5,000
3	4,998	0	0	1	1	0	0	0	0	0	5,000
4	5,000	0	0	0	0	0	0	0	0	0	5,000
5+6	4,999	0	0	1	0	0	0	0	0	0	5,000
7+8	4,997	0	0	3	0	0	0	0	0	0	5,000
9+10	4,997	0	0	3	0	0	0	0	0	0	5,000
Totals	32,103	0	0	11	1	0	0	0	0	0	32,115

partial sex-mosaics, and one half-and-half gynandromorph were found. The male parts of the latter had lost the maternal X chromosome.

The interesting point brought out by these two experiments is the fact that no mosaic flies, other than the sex-mosaics, were produced by X-raying the female germ cells. The significance of this will be discussed in a later section.

#### *Treated males*

For the treatment of the male germ cells, two groups of unmated scute-8 apricot males were at first used. In one group the males were not over eighteen hours old, while in the other group they were five days old. By the time half of the projected experiments had been completed, it was seen that there was no fundamental difference in the results that had been obtained from the two groups. This was true both for the rate and for the character of the  $F_1$  variant flies. It was then decided to combine the results under a single experiment. The tests were continued with males of various ages, but in no test were the males used more than five days old.

The males were given a dosage of 3975 r units, or exactly three times as strong as that administered to the females, and then mated to females of the recessive stock. This stock was balanced to the well-known CIB X chromosome. Both the homozygous females and the heterozygous bar females were mated to the males, but the  $F_1$  bar females were of no use and were not counted or recorded.

Half of the males from any lot were treated, and half were not treated, the latter serving as controls. The periodic transfer method was employed, and for each culture twenty males and from thirty to forty females were placed in a food vial. At the end of each transfer period, the flies were etherized and the males and females separated. For the next period the males were again mated to virgin females, while the fertilized females were placed in a bottle containing food rich in yeast. At the end of six or seven days these females were removed from the bottle, and later their offspring in the bottle and its corresponding mating vial were examined and classified as in the previous experiments. It is possible roughly to tell, by this method, the age of the sperm (at the time of treatment) that fertilized a particular set of eggs, for all of the offspring of any given period came from eggs that were inseminated by sperm received by the females over a definite period of time.

In table 5 are given the data that were obtained in this series of experiments. The total number of flies that had come from eggs fertilized by treated X-bearing sperm was 20,303. In the last column are also given the number of flies obtained for each of the seven periods. The numbers vary from 244 for the sixth period to 6051 for the third period. This varia-

TABLE 5

*Scute-8 apricot males, treated at 3975 r units, and crossed to y w c<sub>v</sub> m f-3/C 1 B females.*

DAYS	APRICOT FEMALES	ABERRANT FLIES		MOSAIC FLIES				LETHAL MUTATIONS					TOTALS
		y w <sup>a</sup> FEMALES	w c <sub>v</sub> m f <sub>3</sub> MALES	MOAIC MALES	GRAY- YELLOW FEMALES	SEX-MOSAICS PARTIAL	SEX-MOSAICS HALF	w	w-f <sub>a</sub>	f <sub>a</sub>	m	f	
1	4,690	33	6	6	7	2	2	8	1	2	13	0	4,770
2	5,655	14	14	2	5	1	2	8	2	2	6	2	5,713
3	5,976	20	11	4	4	6	6	9	5	2	5	3	6,051
4	2,620	11	5	0	1	0	2	4	2	0	2	1	2,648
5+6	425	5	0	0	0	0	0	2	0	1	1	1	435
7+8	239	2	2	0	0	0	0	0	0	1	0	0	244
9+10	441	1	0	0	0	0	0	0	0	0	0	0	442
Totals	20,046	86	38	12	17	9	12	31	10	8	27	7	20,303

tion is due in part to the fact that the same number of cultures were not examined for each of the several periods, and in part to the fact that irradiation reduces the fertility, especially when applied to certain stages of the germ-cell cycle. There were forty-nine cultures carried through the entire seven periods, and a variable number for the first four periods. The best way in which to show the effects of irradiation on fertility is to calculate the average number of F<sub>1</sub> flies per culture. These calculations give the following results for the seven periods: first (114), 41.8; second (97) 58.8; third (105), 57.6; fourth (97), 27.3; fifth (49), 8.8; sixth (49), 4.9; seventh (49), 9.0. The figures in parentheses indicate the number of cultures examined.

In general, X-rayed males produce distinctly fewer offspring than untreated males. This is evidenced by the much larger number of offspring obtained from the corresponding control cultures. The average numbers of flies per control cultures for the seven periods were 285, 286, 251, 244, 271, 221, 208, respectively. In addition to this general reduction in fertility, there was also noted a differential effect for certain stages of the germ cells. The detection of the selective effect of X-rays was made possible by the periodic remating of the treated males to virgin females. As the figures given in the last paragraph show, the flies were less fertile on the first day than on either of the two succeeding days. This must be due to the effect of irradiation on the sperm cells that were the oldest at the time of treatment. On the fourth day there is a decided drop in fertility. In fact, the number of offspring produced is less than half of that for either the second or the third day. The three remaining periods show a still greater decrease, with the lowest average on the sixth period. The increase in the average for the seventh or last period above that for the sixth indicates, possibly, a slight recovery in fertility.

The extreme reduction in fertility, extending from the fourth day through the tenth day, must be due to the destructive effect of the irradiation on immature germ cells. The periodic remating of the treated males to a surplus of virgin females results in the rapid using up of the germ cells that were nearest to maturity at the time of treatment. The destruction by irradiation of the immature cells is certain to bring on a period of great infertility, or even that of sterility. This is in accord with the facts developed in the field of radiotherapy where it has long been known that immature cells are much more radiosensitive than are old cells (DESJARDINS 1932).

We may now consider the variant flies that were secured from the series of treated males, as shown in table 5. There were 246 variants (omitting the partial sex-mosaics, and correcting for controls) that could be attributed to the effects of X-rays. These occurred among 20,303  $F_1$  flies that had been derived from eggs fertilized by treated X-bearing sperms. This gives an average of one variant in every eighty-two flies. That is to say, one out of every eighty-two X chromosomes, treated with a dose of 3975 r units, resulted in producing some type of chromosome irregularity detectable among the  $F_1$  offspring.

Of the eighty-six yellow apricot aberrant females, at least eighty-four (two were found among the controls, table 6) must have been the result of the irradiation breaking off the left end of the treated scute-8 apricot chromosome. This gives a rate of about one in every 240 flies. The rate of their appearance varies for the different days, being one in 144, 408, 302, 240, 87, 122, and 442 flies respectively, for the seven periods included in the table.

TABLE 6  
*Scute-8 apricot males, untreated, crossed to  $y w c_v m f-3/C 1 B$  females.*

DAYS	APRICOT FEMALES	ABERRANT FLIES		MOSAIC FLIES			LETHAL MUTATIONS	TOTALS
		$y w^a$ FEMALES	$w c_v m f_1$ MALES	GRAY- YELLOW FEMALES	SEX-MOSAICS PARTIAL	HALF		
1	5,132	1	0	0	0	0	0	5,133
2	6,007	0	0	0	1	0	0	6,008
3	6,049	0	0	0	1	0	0	6,050
4	3,170	0	0	0	1	0	0	3,171
5+6	1,497	1	0	0	2	0	0	1,500
7+8	1,500	0	0	0	0	0	0	1,500
9+10	1,499	0	0	0	1	0	0	1,500
Totals	24,854	2	0	0	6	0	0	24,862

There were exactly fifty aberrant males produced, at the rate of one in about every 406 flies. The aberrant male carries the untreated X and a treated X chromosome that has been deleted, that is, the middle section has been eliminated. It usually has gray body color, but shows the four

remaining recessive characters—white, crossveinless, miniature, and forked-3. However, twelve of these fifty males were mosaic for gray and yellow. The significance and a possible explanation of their mosaic condition will be discussed in the next section.

The next group of variants represents one of the classes with which this study is primarily concerned, namely, gray-yellow mosaic females. There were seventeen such females obtained, seven appearing on the first day, five on the second, four on the third, and one on the fourth. This class did not appear among the offspring from treated females (tables 1 and 3). These flies are characterized by having approximately one-half of the body gray and the other half yellow.

Twenty-one sex-mosaics were found, and of these nine were of the partial type, and the other twelve were typical half-and-half gynandromorphs. In each of these twelve flies the male parts had lost the treated X chromosome, or part thereof, at the first somatic division.

The last group of variants includes the so-called lethal mutation class. These represent deficiencies at one or more of the several marked loci indicated in the table. There were eighty-three such cases, of which thirty-one occurred at the locus of white, ten involving the loci of both white and facet (Notch), eight at facet, twenty-seven at miniature, and seven at forked. The writer has elsewhere discussed this type of variant fly (PATTERSON 1932c), but the point of interest here is that nine of these cases were "fractionals," that is, only a part of the fly showed the deficiency. For example, the fly would have one miniature wing and one normal wing. The number of these mosaics, occurring at the different loci, was as follows: four at white, one at facet, three at miniature, and one at forked.

The control flies for this experiment are given in table 6. The six partial sex-mosaics require no further consideration. The only other variant flies were two aberrant yellow-apricot females. Both of these had resulted from spontaneous breaks that had eliminated the left end of the scute-8 apricot chromosome. There were no lethal mutations found at any of the marked loci.

#### TYPES OF MOSAICS

##### *Sex-mosaics or gynandromorphs*

The experimental results presented above have brought out the fact that several different kinds of mosaic flies are produced, including the sex-mosaics. Seventy-two sex-mosaics were detected, and fifty of these belonged to what we have termed partial sex-mosaics. This type of fly is almost entirely female, but has a limited area of male tissue, such as a sex-comb, male genitalia, or male coloration on a part of the tip of the abdomen. In this series these mosaics occurred with equal frequency among the

control and experimental series. Among 80,918 flies of the experimental series, twenty-four partials were found. This gives a rate of one in every 3371 flies. Among 87,479 flies of the control series, twenty-six partials occurred, and this gives a rate of one in every 3364 flies. As stated above, these mosaics are usually not produced by X-rays, but are the result of spontaneous elimination of one of the X chromosomes in a relatively late somatic division. Occasionally, the elimination may occur at one of the early cleavage divisions, and if this should take place at the first division, there would be produced a half-and-half gynandromorph indistinguishable from one caused by irradiation.

In the treated female series, eight typical half-and-half gynandromorphs were found. The male parts of seven of these had lost the maternal or treated X chromosome, while those of one had lost the untreated or paternal X. One might infer that the latter case had occurred spontaneously, since the untreated X was missing, but, as I have elsewhere shown, there is an indirect effect of irradiation on the cytoplasm of treated eggs which not infrequently causes the elimination of the X chromosome introduced by the sperm (PATTERSON 1931). However, since two half-and-half gynandromorphs were found among the control flies, it is very probable that one or two of the cases found in the experimental series were also the result of spontaneous elimination.

In the treated male series twelve half-and-half gynandromorphs appeared, but none was found among the control series. The male parts of each of these flies had lost all or a part of the paternal or treated X chromosome. In five of the cases an extensive deletion had resulted in the elimination of the middle region of the X, so that the male parts were gray, but with the exception of yellow, they showed the other recessive markers of the untreated X. The male half of such gynandromorphs is the counterpart of the aberrant male, which is produced by a similar deletion that affects the single-strand stage of the chromosome. In the case of the gynandromorph, the deletion takes place in the two-strand stage, and affects only one of the two strands. Consequently, the initial somatic division gives the first opportunity for the deleted and non-deleted elements to separate into separate cells.

On this basis, one can readily understand why treating the female germ cell does not result in the formation of a gynandromorph that carries in its male tissue a broken or deleted X chromosome, because at the time of treatment the two-strand stage of the gametic chromosome has not been reached. X-raying the eggs, therefore, usually results in the production of aberrant males and females, although a gynandromorph is occasionally formed as a result of the elimination of one of the daughter X chromosomes at the first cleavage division. Gynandromorphs derived from X-rayed

mothers should not possess male parts carrying a broken or deleted X chromosome. I have, however, reported three such cases and attributed the breaks to the effects of X-radiation (PATTERSON 1931a). Two of these cases were due to the elimination of the X chromosome to which the *Theta* fragment was attached, but, since this phenomenon may occur spontaneously, these two cases do not constitute proof that the effect was due to X-rays. The third case represented one in which the X chromosome was broken at a point lying to the left of forked. It now seems probable that this case resulted from a spontaneous break which occurred after the beginning of maturation and before the first cleavage took place. This would give time enough for a break to take place after the split in the gametic element had occurred. This interpretation is strengthened by the recent discovery of a gynandromorph among untreated flies that had male parts with a broken maternal X chromosome.

At rare intervals gynandromorphs appear that cannot be explained on the simple elimination hypothesis, but can be accounted for on the basis of binucleated eggs. Such cases were first reported by MORGAN and BRIDGES (1919), and have since been discussed by L. V. MORGAN (1929). STERN and SEKIGUTI (1931) have described a mosaic male derived from a binucleated egg, but they were not able to determine whether the two nuclei were the partial products of the maturation divisions of an originally single egg nucleus, or whether the oocyte was binucleated from the first.

### *Mosaic females*

Several different types of mosaic females are produced by breaks induced in one of the strands of the two-strand stage of the X chromosome of the sperm. One type is formed as a result of a single break, and is represented by the gray-yellow half-and-half mosaics. The fact that they are found in the treated male series, and not in the treated female series, at once suggests that they arise from sperm in which the gametic X chromosome is already split at the time of irradiation. Otherwise, it would be difficult to explain their mosaic condition. They appeared at the rate of one in every 683, 1142, 1513, and 2648 flies, respectively, for the first four days or periods (table 5). These numbers, however, are not large enough to show whether the difference in rate for the different days is significant. It is of interest to note that they appeared at a decreasing frequency for the successive days, but when all types of mosaics arising from split chromosomes are considered, there is little or no indication of a decreasing trend, the rate being one in every 530, 571, 503, and 882, respectively, for the first four periods.

The second type of mosaic female is found among the "lethal mutations" class of flies. These were found in the treated male series, and all appeared



within the first three periods. It is not always possible to determine by breeding tests on fractionals whether the variation is due to a point-mutation, or to a deletion, because cells carrying the affected chromosome may not have been included within the germ glands. Nevertheless, they are important in indicating the split condition of the X chromosome in the sperm at the time of treatment.

### *Eversporting types of mosaics*

Another class of variant produced by X-rays includes some of the so-called eversporting mosaics. The most common type of this class is caused by a chromosome rearrangement involving the locus of white, so that flies heterozygous for red and white show mottled-eyes. Sometimes adjacent genes are involved, especially the locus of facet, resulting in the production of mottled-eyed and variable notched-wing flies. Many of these cases have not as yet received an adequate explanation, but the writer has found two cases that are due to unstable translocations. One of these has been worked out both cytologically and genetically. It was found that a piece of about six map units of the left end of the *Theta* X chromosome had been broken off and translocated to a fourth chromosome. This piece carried the normal genes for white and facet, and its occasional loss from the attached fourth during somatogenesis resulted in the production of mottled-eyes and variable notched wings (PATTERSON 1932b). Still another case belonging to this category was found several years ago, and was partly worked out before the stock was lost. In this stock the flies heterozygous for gray and yellow showed yellow spots scattered over the body. Linkage tests showed that a small piece containing the normal gene for yellow had been broken off and translocated to a third chromosome. The loss of this piece during development produced the yellow spots.

This same type of eversporting mosaicism may occur as a result of spontaneous elimination in untreated material. A very good example is found in the scute-8 apricot stock. If homozygous females are crossed to yellow white males, about one in every sixty-six  $F_1$  females shows one or more yellow spots on the body (4952 examined). Irradiation does not appreciably increase this rate, for in the treated series, one in every fifty-seven  $F_1$  females had yellow spots (2165 examined). That the spotting is not due to a mutable gene is indicated by the fact that the scute-8  $F_1$  males are never spotted.

This case may be brought into line with the three cited above if we assume that the left end of the scute-8 X is occasionally lost during the course of development. This interpretation is made probable by a few tests that have been made with  $F_1$  females. Three non-spotted and five spotted females were backcrossed to yellow white males. The three non-spotted

and four of the spotted females gave no spotted offspring. The fifth spotted female gave eighteen  $F_2$  females, all showing yellow spots, but failed to yield any scute-8 apricot males. The stock established from this line continues to breed in this same manner. The loss of the left end of the scute-8 X creates a deficiency and hence, since it occurs in every X, the scute-8 apricot male zygote is non-viable. That the break is not followed by the translocation of the piece to one of the autosomes is made certain by the fact that none of the white-eyed females in the cultures are ever gray.

### *Mosaic males*

We have already mentioned the fact that mosaic males are found in the treated series. These flies display various degrees of coarse and fine mosaicism, from types showing half the body gray and half yellow, with a sharp line of demarcation between the two halves, to types showing a fine mosaic of small gray and yellow areas (pepper and salt types). These flies arise as aberrant males and are therefore hyperploid. They have the untreated X, marked with the five recessive genes, and a deleted fragment of the treated X chromosome. The mosaic condition applies only to the yellow locus, for the other four recessives always show. The mosaic can be explained on the same basis offered above to account for some of the eversporting types of females, that is, the left or gray end of the deleted X is sometimes lost during the cleavage stages. If the elimination occurs once and at the first division, a half-and-half mosaic male would result, but if it took place at a later division, a male with a yellow spot would be produced. The fine mosaic type would result from several eliminations occurring in the late cleavage stages. It is possible that some of the mosaic males, as well as some of the aberrant males, have arisen from treated gametes in which the gray end had been translocated to another chromosome, rather than by a reattachment with the right-hand end of the deleted element. This cannot be determined genetically because of the fact that such males have no Y chromosome and are consequently sterile.

There are other types of mosaics than those obtained in this series of experiments. Thus by X-raying larval stages, heterozygous for various mutant characters, it is possible to produce "somatic mutations" that appear in the adult fly as mosaic or variant areas (PATTERSON 1929a, 1929b). Some of these areas are the result of induced point mutations, but most of them have been brought about through the elimination of a section of the chromosome that carried the dominant genes. The writer has also reported the finding of mosaics that resulted from somatic segregation of chromosomes, caused by X-raying egg and larval stages, heterozygous for yellow and singed (PATTERSON 1929c).

Mosaic flies caused by the loss of autosomal material are also known to oc-

cur. BRIDGES (1921) showed that the small fourth may be lost through non-disjunction. This gives rise to haplo-IV flies that have fine delicate bristles, a condition known as "Diminished." Recently MOHR (1932) has described and tested a fly that was normal on one side and haplo-IV on the other. DOBZHANSKY (1932) has reported a case (his translocation H) in which a small piece, including the locus of purple, had been broken out of a II chromosome and translocated to a Y chromosome. He obtained two females that were deficient at the locus of purple, showing that such hypoploid females may be viable. Finally, STERN (1927) has described two cases of mosaic spotting, caused by spontaneous eliminations of parts of the III chromosome. In one case the tissues of the mosaic spot were deficient for the left arm of the third, and in the other for half of the right arm of this same chromosome.

In general, however, the loss of either one of the two large autosomes, or even of more than a small piece thereof, is fatal. With respect therefore to the viability of the fly, the elimination of autosomal material stands out in sharp contrast to the loss of X-chromosomal material. This fundamental difference may have been the outcome of a long selective process. Through the evolution of the sex-determining mechanism, the fly has become adapted to the absence of one of the X chromosomes (but not to the absence of a large autosome) and consequently one can bring about the elimination of a very considerable portion of one of these chromosomes in an XX zygote without causing the death of the individual.

#### DISCUSSION AND CONCLUSIONS

In the majority of cases, the fundamental cause underlying the mechanism of mosaic formation is the process of chromatin elimination. Another important fact concerning mosaic formation relates to the condition of the chromosome at the time the irradiation is applied. The general rule is that if the chromosome is not split, a break or deletion will produce an aberrant male or female, depending upon the particular region eliminated; but if the chromosome has already split and is in the two-strand stage, a break or deletion in one of these strands will give rise to a mosaic fly. To a certain extent, one can obtain a desired type of variant by applying the irradiation to certain stages of the germ cells or larvae.

The results obtained in this and previously reported experiments indicate that, with few exceptions, breaks and deletions induced in the egg chromosome by X-raying virgin females give rise to aberrant flies only. We should expect this to be the case from the work of HUETTNER (1924) on maturation and fertilization. He found that the polar bodies are not given off until after the sperm has entered the egg. The ovarian egg has a large, reticular nucleus, but in eggs about ready to be fertilized the meta-

phase spindle of the first polar body is present. In treating the egg in the ovary, the irradiation is applied to chromosomes that in the main are in the tetrad or pretetrad condition, and, consequently, a deficiency induced in any chromosome will affect both of its potential strands. A mature egg that retains the broken element will, upon fertilization, develop into a fly that carries the deficiency in all of its cells, that is, it will be of the aberrant type. If mosaics with deficient chromosomes are produced at all, the break must have taken place in those eggs that had formed the first polar spindle, where there might be a chance for the gametic split to occur.

The production of mosaics from treated eggs is confined mainly to sex-mosaics of the half-and-half type. Such mosaics or gynandromorphs do not carry a broken chromosome (one exception, explained above), but are the result of the elimination of an entire somatic chromosome, usually at the first cleavage division, and therefore following maturation and after the gametic split had occurred. It has been found that the rate at which half-and-half gynandromorphs appear among  $F_1$  flies derived from treated females is increased about three times over that at which they are found among the controls. It has also been observed (MORGAN and BRIDGES 1919, PATTERSON 1931) that the maternal and paternal X chromosomes are eliminated with equal frequency in gynandromorphs derived from untreated flies. In the case of gynandromorphs from treated mothers, the maternal and paternal chromosomes are likewise lost with about equal frequency.

This fact lead the writer (1931, p. 199) to suggest that at least part of the effect of irradiation on elimination must be indirect, probably operating through the cytoplasm of the egg. If this suggestion is valid, then the cytoplasm must eventually recover from this effect. In the present series of experiments little or no evidence was found which indicated that treating the egg increased the type of sex-mosaic which had male areas of less than half the size of the body. This type of gynandromorph occurred as frequently among the control flies as among the experimental flies. However, in some of the previously conducted experiments, especially when the *Theta* stock was used as the treated parent, evidence was found indicating that the effect persisted beyond the first cleavage division, for here the partial sex-mosaics were significantly increased as a result of the treatment.

If the cause of the elimination is brought about through the absorption of the rays by the cytoplasm, there might be a recovery from the effect causing elimination as development progressed. Recovery from the effects of radiation by organisms has been reported in the literature. Thus BOVIE and KLEIN (1918), and BOVIE and DALAND (1923) showed that paramecia, sensitized to heat by exposure to fluorite radiation, recover from the effects of the radiation after about five hours, and HANCE (1926) found that X-

radiation of these same organisms brings about a slight but constant depression in the division rate from which the organisms recover after from two to five days.

Irradiating the sperm cells by treating adult males results in the production, not only of aberrant flies, but also of mosaics, including gynandromorphs. But the increase in gynandromorphs over that found in controls is restricted to the half-and-half type. The partial sex-mosaics show no appreciable increase as a result of the irradiation. I have obtained a total of one hundred and nineteen half-and-half gynandromorphs among flies derived from treated males. Their distribution with reference to the elimination of the treated or untreated X chromosome is entirely different from that reported above for the treated female series. One hundred and thirteen of these had lost the treated X (or part) from their male parts, and only six had lost the untreated or maternal X. Undoubtedly, some half dozen of those in which the treated X was found to be missing were not caused by the irradiation but were the result of spontaneous eliminations. This leaves one hundred and seven cases that can safely be attributed to the direct effects of irradiation. In forty-five flies the male parts carried, in addition to the marked maternal X, a broken or deleted paternal X. In all such cases the irradiation must have affected only one of the two gametic strands. The remaining sixty-two cases gave the appearance of having lost one entire gametic strand of the treated X chromosome. This was proved to be true in some cases by the use of the *Theta* stock in which the gray fragment at the right end of the X chromosome served as a marker. In cases derived from treated non-*Theta* stock, it is possible that in some instances the treated X had been broken at a point lying to the right of the normal allelomorph of the last marker and the right-hand end of the chromosome, for in this event the presence of the broken chromosome could not be detected.

The mosaic females derived from treated sperm fall into two classes, the gray-yellow class and the fractional or lethal class. Both of these classes are important, because both indicate that the breaks which caused them took place in only one of the strands of the two-strand stage. This raises the question as to why X-rayed sperm give rise to both aberrant and mosaic flies. The ratio of aberrant females to mosaic females is about six to one. With reference to the condition of the gametic chromosome at the time of treatment, there are three possibilities, as follows: (a) The chromosome may not be split in any of the sperm, (b) it may be split in all sperm and (c) it may be split in some sperm but not in others. We may now consider what would follow on the basis of each of these assumptions.

If we assume that the chromosome is not split in any of the male gametes at the time of treatment, the appearance of aberrant flies can easily be

accounted for, but in order to explain the appearance of mosaic females, it would be necessary further to postulate some kind of delayed radiation effect which would bring about the break in one of the strands after the split had occurred. Aside from the fact that we have been unable to obtain any experimental proof of a delayed effect in breakage, there is the further objection that no such effect occurs in the egg chromosome, which is known to be in the one-strand stage at the time of treatment.

The second suggested possibility that the gametic split is present in all fully formed sperm also meets with a serious objection. It is true that some cytologists hold the view that the gametic split occurs in the prophase stages of the preceding division; nevertheless, the genetic evidence is not in harmony with that assumption. On the basis of this suggestion it would be necessary to assume that radiation sometimes breaks one strand and sometimes both strands. If the break is the result of a "direct electron hit," it would be difficult to explain the fact that in about six times out of seven both strands are hit simultaneously.

The simplest explanation to account for the facts of breakage is implied in the third possibility, for the genetic evidence indicates that the gametic split is present in only about one out of every seven fully formed sperms. Our complete lack of knowledge with reference to the exact nature of the effect of radiation in producing breaks in the chromosomes makes it difficult to decide definitely between these three alternatives. In view of all of the facts, however, the writer regards the third alternative as the most probable.

Another point of interest relates to the fate of the broken-off or eliminated piece of the chromosome. In all probability this piece simply disintegrates in the cytoplasm, although the possibility of its loss through the mechanism of a translocation must be considered. Obviously, such aberrant flies as the yellow apricot females, derived from treated male gametes, could not have arisen through a translocation of a part of the broken scute-8 chromosome to one of the autosomes. The irradiation must produce such translocations, but these can not be detected by examination of the  $F_1$  flies, for the deficiency created at the locus of yellow in the broken X would still be covered by its normal allelomorph in the translocated fragment on the autosome.

The corresponding yellow apricot females, derived from treated female germ cells, might possibly arise from translocations. For here the break is induced in prematuration chromosomes and should a translocation occur there would be an equal chance that the resulting gametes would fail to receive the translocated piece. Such gametes upon fertilization by yellow X-bearing sperms would produce yellow aberrant females. But if this were the method, then there would be an equal number of gray hyperploid

females produced. It should be possible to detect at least some of these postulated females without having to resort to breeding tests. In over sixty thousand  $F_1$  gray females from cultures that yielded sixty yellow aberrant females, not a single hyperploid was detected, although the flies were carefully examined with the view of detecting such variants. In view of these facts, it is very probably that the yellow aberrant females from treated mothers have been produced by a simple break, followed by the disintegration of the broken-off piece.

The class of gray-yellow mosaic females might possibly come from induced translocations. These flies are derived from treated sperm, and if the broken-off fragment from one of the strands were to become attached to an autosomal strand, it might segregate into the future gray part of the fly at the first somatic division. If this should happen, some of the sons of those mosaic females yielding gray offspring would reveal the presence of the translocated piece. Seven of the tested mosaic females gave numbers of gray offspring large enough to make the test adequate, but in no case were males carrying a translocated piece obtained. Such a male would have gray body color, but would show the remaining recessive characters present in the marked maternal X chromosome. This evidence supports the conclusions that the gray-yellow mosaic females result from simple breaks in the treated X chromosome.

#### SUMMARY

There is reported in the paper a series of new experiments which had as their object a more exact determination of the nature of the effects of X-radiation on the production of mosaic flies by breaks in the X chromosome. The scute-8 apricot stock used in the experiment is characterized by having an X chromosome with a long inverted section. This condition made it possible to obtain considerable numbers of aberrant and mosaic flies by breaking off the left end of the X chromosome—a result that is rarely attained when a normal X chromosome is treated. The main points brought out in the experiments are summarized in the following paragraphs.

1. The main cause underlying the production of mosaic flies is chromatin elimination. The elimination may involve the entire X chromosome but frequently includes only a part of that chromosome.

2. The type of fly resulting from breakage depends upon the condition of the chromosome at the time the break is induced. If the gametic element is not split at this time, the resulting fly will be of the aberrant type, and will reveal the deficiency in all of its tissues; but if the gametic split is present, the elimination of all or part of one of the strands will produce a mosaic individual.

3. It was found that if female germ cells were treated by X-raying vir-

gins, only the aberrant type of fly developed, following breakage of the X chromosome. This was taken to mean that the gametic split had not developed at the time of raying. The number of sex mosaics found among F<sub>1</sub> flies from treated mothers is significantly increased over that occurring among the controls. There is no conclusive evidence that these sex-mosaics carry a broken X chromosome, but they do show that their male parts lose the paternal X with the same frequency as the maternal X. This lead to the suggestion that the effect of radiation on the elimination of the X chromosome is in part indirect, probably operating through the cytoplasm.

4. X-raying mature sperm cells result in the production of both mosaic and aberrant flies, in the ratio of one to six. This indicates that at the time of treatment the X chromosome is split in about one out of every seven sperms. Treating the male germ cells also increases the number of gynandromorphs. Over forty percent of these carry a broken X chromosome in their male parts, but most, if not all, of the remaining sex-mosaics have lost half the treated paternal X at the first somatic division. There is no evidence that the treated sperm cell affects the elimination of the maternal X chromosome brought into the zygote through the egg.

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# SEGMENTAL INTERCHANGE IN CHROMOSOMES OF TRADESCANTIA

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Chromosome ring formation has been found in a number of different genera. In certain species of *Oenothera* and of *Hypericum* and in the monotypic genus *Rhoeo*, all the chromosomes may be united in a single ring or chain at meiosis. Ring formation is maintained in these plants by a system of balanced lethals. In *Datura*, *Zea*, *Pisum* and *Campanula* rings of four or more chromosomes have been described. In most species with either large or small rings there is considerable pollen sterility due to non-disjunction at meiosis.

BELLING's (1927) hypothesis that ring formation is caused by segmental interchange between non-homologous chromosomes has been confirmed by the cytological and genetic evidence in *Oenothera* hybrids (CLELAND and BLAKESLEE 1931, EMERSON and STURTEVANT 1931) and in *Zea* (MCCLINTOCK 1930, BURNHAM 1930, 1932, BRINK and COOPER 1931, 1932) and from a study of chromosome arrangement in *Rhoeo* (SAX 1931). The behavior of reciprocal translocations in *Drosophila* (STURTEVANT and DOBZHANSKY 1930) is also in accord with BELLING's hypothesis.

The different types of chromosome configurations in the rings or chains is of interest in connection with the mechanism of chromosome segregation at meiosis. Interlocking of bivalents and rings has been found in several ring-forming genera, and the relations of these locked pairs of bivalents have an important bearing on the nature of chiasma formation. Little is known concerning the mechanism involved in producing segmental interchange. Chromosome ring formation in *Tradescantia* provides an opportunity for a more complete study of these problems.

## CHROMOSOME PAIRING IN NORMAL AND SEGMENTAL INTERCHANGE TRADESCANTIAS

Chromosome pairing at meiosis has been studied in normal diploid plants of *Tradescantia bracteata* Small, *T. edwardsiana* Tharp, *T. reflexa* Raf., and *T. gigantea* Rose; in a tetraploid *T. occidentalis* Britton; in several segmental interchange plants of *T. edwardsiana* and *T. reflexa*; and in *Rhoeo discolor* Hance. The analysis of chromosome configurations is based on aceto-carmines smears. In most cases, a small proportion of aceto-haemotoxylin was added to the aceto-carmines. After the smears were covered, the slides were heated gently and then inverted and slightly

The accompanying Heliotype plate is paid for by the GALTON AND MENDEL MEMORIAL FUND.

## EXPLANATION OF PLATE 1

Photomicrographs of *Tradescantia* chromosomes from aceto-carmines smears. Magnification  $\times 1500$ , except figure 11 which is  $\times 1000$ . The outline of each photograph corresponds to the cell wall. Figures 1 to 9 inclusive are from preparations of *T. edwardsiana* with segmental interchange chromosomes.

FIGURE 1.—Chain of four chromosomes with terminal chiasmata and four bivalents. The orientation of adjacent chromosomes towards opposite poles in the chain will result in regular disjunction and fertile pollen grains. The bivalent below the chain is a double ring with three chiasmata.

FIGURE 2.—Regular disjunction type of chain of four chromosomes with two subterminal chiasmata. The middle chiasma in the chain is almost always terminal.

FIGURE 3.—The chain of four chromosomes is interlocked with a ring bivalent. This chain is also of the disjunctional type and has one subterminal and two terminal chiasmata.

FIGURE 4.—A non-disjunctional type of chain with one subterminal chiasma. The typical bivalent configurations—3 rings and 1 rod—are clearly shown.

FIGURE 5.—A non-disjunctional chain with one subterminal chiasma. Two rod bivalents are shown,—one with a terminal and the other with a subterminal chiasma.

FIGURE 6.—A non-disjunctional chain with two subterminal chiasmata. This characteristic position of the subterminal chiasmata indicates the relative lengths of the segments in the interchange complex.

FIGURE 7.—A ring of four chromosomes with two ring and two rod bivalents. Rings of four chromosomes were found much less frequently than chains.

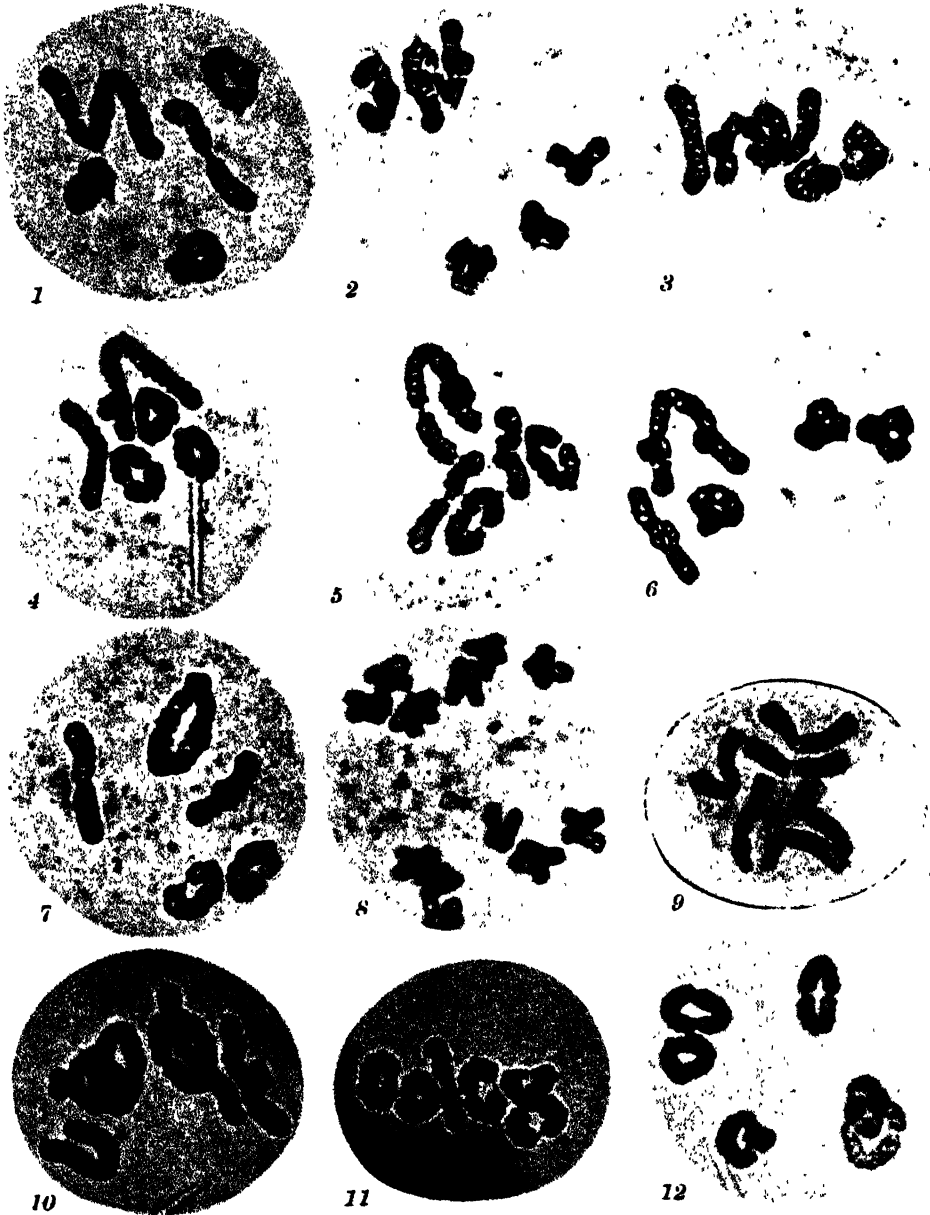
FIGURE 8.—Later anaphase of first meiotic division with 6 chromosomes at each pole. The chromatids of each homologue are attached only in the region of the spindle fiber.

FIGURE 9.—The haploid set of chromosomes in the microspore. Three of the chromosomes are approximately isobrachial, and three are heterobrachial.

FIGURE 10.—Meiotic chromosomes of *T. edwardsiana* 15, with 6 bivalents. Two ring bivalents are interlocked, and a rod bivalent is locked with a ring bivalent.

FIGURE 11.—Meiotic chromosomes of *T. bracteata*. Six bivalents were usually found, but chains and rings were found in rare cases. A ring of four chromosomes is shown at the right.

FIGURE 12.—Meiotic chromosomes of *T. edwardsiana* 15 with interlocked ring bivalents. Interlocked bivalents were found in most of the pollen mother cells of this plant.





pressed on a sheet of filter paper. In this way the excess fixing fluid was removed, and the cells were flattened sufficiently so that the chromosomes could be studied and photographed easily.

The chromosomes of normal diploid *Tradescantias* usually form ring- and rod-shaped figures at meiosis. The homologous chromosomes may be united by terminal or subterminal chiasmata. Occasionally three chiasmata are found in a single bivalent. The average chiasma frequency found in *T. bracteata* was 1.9, in *T. reflexa* 1.8, and in *T. gigantea* 1.7.

Interlocked bivalents (figures 10 and 12) have been found in all diploid species examined. In *T. bracteata*, interlocked bivalents were found in 20 percent of the pollen mother cells; in *T. reflexa* 15 percent, and in *T. gigantea* 40 percent of the pollen mother cells contained interlocked chromosomes.

In all these diploid species there are six pairs of chromosomes of which three have sub-median spindle-fiber constrictions and the other three have approximately median constrictions.

The arrangement of the chromosomes in tetraploid *Tradescantias* is of interest in comparison with the configurations found in segmentally interchanged diploids. An examination of the chromosomes in 73 pollen mother cells of a tetraploid, *T. occidentalis*, showed that about half of the chromosomes were paired as bivalents and about half as quadrivalents. The quadrivalents were found as rings or chains of chromosomes with terminal chiasmata. In about 80 percent of the quadrivalents, adjacent chromosomes passed to opposite poles. The normal tetraploids are fertile and 85-90 percent of the pollen is morphologically perfect.

*T. edwardsiana* is a well-marked and distinctive species, though only recently described. It is apparently limited to the Edwards Plateau in central Texas and morphologically seems to be more closely related to the northern species *T. pilosa* than to any other known species from Texas. Collections were made at two near-by points in the Bull Creek region, near Austin, two plants being collected at the first station and eighteen at the other. Two of the plants from the second station (numbers 2 and 15A) were characterized by four bivalents and a chain or ring of four chromosomes at meiosis. The other 18 plants were apparently normal diploids with 6 bivalent chromosomes. The interchange chromosomes were found as chains of four in most cases. The four chromosomes are united end to end by terminal chiasmata (figure 1) or by terminal and sub-terminal chiasmata (figures 2, 3, 4, 5, 6, and 7). In rare cases each of the three chiasmata in the chain may be subterminal. Closed rings with four chiasmata are comparatively rare (figure 7). The types and frequency of chiasmata found in the interchange complex are shown in table 1.

A total of 648 pollen mother cells was studied. In 550 cells, chains or

TABLE 1  
*Chiasma frequency in interchange complex of T. edwardsiana plant 15A. (Numbers in parentheses represent subterminal chiasmata.)*

3	3(1)	3(2)	3(3)	4	4(1)	4(2)	N
158	228	125	12	6	14	7	550

rings of four chromosomes were found, while in 98 cells only bivalent chromosomes were observed. In four cases there were trivalent-univalent groups of chromosomes. The average chiasma frequency in the chains and rings is only 3.05. Thirty-two percent of the chiasmata are subterminal.

In the chain of four chromosomes, subterminal chiasmata are found between the first and second or between the third and fourth chromosomes (figures 2, 3, 4, 5, and 6) but are rare between the second and third chromosomes. This distribution of the subterminal chiasmata and the prevalence of chains rather than rings indicate that segmental interchange has occurred between heterobrachial chromosomes. The arrangement of the chromosome segments is presumably indicated by figure 13.

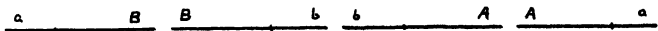


FIGURE 13.

The adjacent chromosomes in the chain may go to opposite poles (figures 1, 2, and 3), forming fertile combinations of chromosomes, or they may go to the same pole (figures 4, 5, 6, and 7), forming sterile combinations due to a deficiency of a chromosome segment. The two types of distribution occur with about equal frequency—266 fertile to 284 sterile.

The proportions of fertile and sterile types of segregation are partly dependent on the number and position of the chiasmata in the chains and rings. The chains and rings with only terminal chiasmata show a ratio of 108 fertile to 56 sterile types of segregation. In chains with one subterminal chiasma, the ratio was 110 fertile to 132 sterile; with two subterminal chiasmata the ratio was 48 fertile to 84 sterile, while the 12 chains or rings with 3 subterminal chiasmata were all of the sterile type. Of the 27 closed rings only 1 was of the fertile type with adjacent chromosomes passing to opposite poles.

TABLE 2  
*Regular and non-disjunctional segregation of chains of 4 chromosomes in relation to the presence of subterminal chiasmata.*

NUMBER SUBTERMINAL CHIASMATA	REGULAR	NON-DISJUNCTIONAL	PERCENT NON-DISJUNCTIONAL
0	108	56	34
1	110	132	55
2	48	84	64
3		12	100

The sterility expected from the observed percentages of the sterile type of segregation in the ring or chain is about 50 percent. But in about 15 percent of the pollen mother cells of plants 15A, only bivalents are found, and the segregation of the two bivalents of the interchange complex must be considered. If the homologous chromosomes of each of these two bivalents pass at random to either pole, the percentage of sterility expected will not be changed. The percentage of fertile types of segregation is greatest in chains of chromosomes with no terminal chiasmata, so with even greater freedom of assortment in two bivalents, there might be an excess of fertile types among the bivalents which would increase the fertility somewhat above 50 percent.

Pollen sterility is also dependent on chromosome distribution. In about 90 percent of the pollen mother cells, six chromosomes were found at each pole at telophase (figure 8), but in about 10 percent of the cells the distribution was 5-7. The microspores with 5 chromosomes apparently do not develop; but 7 chromosomes have been observed in microspore divisions, indicating that these types may form good pollen grains. Lagging univalents were also found which would result in chromosome deficiencies. An examination of 600 pollen mother cells showed lagging univalents in 30 cases. Unequal chromosome distribution and lagging univalents should produce about 7 percent pollen sterility.

Pollen counts from anthers about to open indicate about 50 percent sterility—642 apparently normal pollen grains and 538 imperfect ones. In view of the variations in chromosome segregation and pollen sterility, the correlation between chromosome behavior and pollen sterility is very close.

Interlocking of chromosomes was found in the segmental interchange plants as well as in normal diploids. In about 10 percent of the pollen mother cells of plants 15A, the chain or ring was interlocked with a bivalent. A ring bivalent locked on a chain is shown in figure 3. Occasionally two bivalents are locked on a chain or ring. Interlocking of bivalents was also found, but occurs in only 5 percent of the pollen mother cells. The total amount of interlocking between non-homologous chromosomes in *T. edwardsiana* 15A is less than in other normal species and much less than in some of the plants of *T. edwardsiana* with 6 bivalents.

A plant of *T. edwardsiana* (15) with 6 bivalents was examined for comparison with the segmental interchange plant (15A). The number and types of chiasmata found in the 6 bivalents of plant 15 and in the 4 bivalents of plant 15A are shown in table 3.

The average chiasma frequency is the same for the bivalents of both plants, although the number of subterminal chiasmata is greater in 15A (33 percent) than in 15 (19 percent). In the rings and chains of plant 15A,



TABLE 3

*Number of chiasmata in bivalents. (Number in parentheses are subterminal.)*

		0	1	(1)	2	2(1)	2(2)	3(1)	N	AVERAGE
<i>T. edwardsiana</i>	15	2	120	51	303	124	4	14	618	1.7
<i>T. edwardsiana</i>	15A	4	28	22	47	47	8	12	168	1.7

the percentage of subterminal chiasmata is also about 33 percent, but the chiasma frequency is about 3, or 1.5 per bivalent.

Only 2 interlocked bivalents were found in 42 pollen mother cells of plant 15A, while, in plant 15, there were 148 interlocked bivalents in 103 pollen mother cells. Two interlocked ring bivalents and a ring locked around a rod are shown in figure 10. Another pollen mother cell of plant 15, with interlocked bivalents is shown in figure 12. In a few cases as many as 5 bivalents were found interlocked, and interlocking of three bivalents was found frequently. Occasionally a ring or rod bivalent was locked with a double ring bivalent, but it was impossible to determine whether or not the locking involved the internode with the fiber constriction. There was about 30 percent pollen sterility in plant 15, when grown in the greenhouse, which might indicate that the chromosome behavior is not entirely normal.

Chromosome rings and chains have also been found in a single plant of *T. reflexa*. This species is usually a tetraploid in the northern states (ANDERSON and DIEHL 1932), but several diploid plants from eastern Michigan were obtained through the courtesy of the Botanical Garden of the UNIVERSITY OF MICHIGAN. One of them, a peculiar looking plant with light, gray-blue flowers, had been collected at Dexter, Michigan. Like the two plants of *T. edwardsiana*, it was characterized by rings and chains of 4 at the reduction division. The average chiasma frequency was found to be 1.76 for the bivalent chromosomes and 3.84 for the rings and chains. Interlocking of bivalents was rare, only one case in 107 pollen mother cells, but interlocking of the ring or chain with a bivalent was observed in 15 pollen mother cells out of the 107.

In this Dexter plant, the rings were much more frequent than chains. Adjacent chromosomes were oriented towards the same pole in 83 percent of the 107 cells studied. About 90 percent sterility might be expected with such a segregation if the interchange complex is typical, but the pollen sterility is usually relatively low. Pollen counts from 98 different flowers, and on 16 different days, showed from 10 to almost 100 percent fertility. The general average of all pollen counts (from flowers collected on different dates) was 65 percent morphologically perfect pollen. Counts from individual anthers showed uniformity for individual flowers.

The chromosomes of the interchange complex in *T. reflexa* may be united by either terminal or subterminal chiasmata. A chain of four chromosomes united by terminal chiasmata is shown in figure 14A. In about 40 percent of the rings or chains, one or more subterminal chiasmata were observed. A typical ring with a subterminal chiasma is shown in figure 14B. This chiasma is peculiar in that it appears to form a loop, and the adjacent chromosome segments are at different levels in some cases. The asymmetrical arrangement of the chromosomes was found in many figures, and the position of the subterminal chiasma is typical. In many of the microspore divisions, one chromosome was considerably longer than the others.

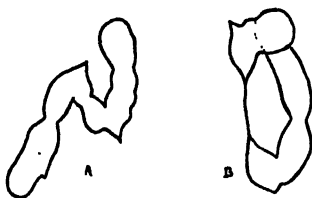


FIGURE 14.

In certain diploid species with 6 bivalents at meiosis, rings or chains of four chromosomes are very rarely found. Several such figures have been found in *T. edwardsiana* and in *T. bracteata*. A ring of four chromosomes from *T. bracteata* is shown in figure 11. The rings may be either double loops or circles. They seem to be essentially the same as those found in segmental interchange plants.

The rings and chains of 12 chromosomes in the closely related genus *Rhoeo* have been studied for a comparison with the rings of 4 in *Tradescantia*. According to DARLINGTON (1929), non-disjunction in the 12 chromosomes of *Rhoeo* occurs in about half of the pollen mother cells. One of the writers (SAX 1931) also found about 50 percent non-disjunction, but the counts were not based on many figures. According to GAIRDNER and DARLINGTON (1932), non-disjunction is about 30 percent in *Rhoeo*, but no data or references are given in support of this conclusion.

A careful study of 100 pollen mother cells of *Rhoeo discolor* showed that all 12 adjacent chromosomes were oriented towards opposite poles in only 21 cells. Single non-disjunction was found in 39 cells, double non-disjunction on opposite sides of the spindle was found in 30 cells, and double non-disjunction on the same side was found in 9 cells. Thus, in nearly 80 percent of the cells, there was non-disjunction in the ring or chain of 12 chromosomes. This amount of non-disjunction should result in about 80 percent pollen sterility. The pollen sterility actually found in an examination of about 1000 pollen grains was 86 percent, which is in accord with previous observations (SAX 1931).

The amount of non-disjunction is greater in rings than in chains. In 31 rings, 27 had double non-disjunction, and 2 had quadruple non-disjunction. Only 3 of the 31 rings were regular in chromosome arrangement. In 19 single chains 10 were regular; 8 had single non-disjunction, and 1 cell had double non-disjunction. The cases of single non-disjunction are, of course, accompanied by a second one if the chromosome distribution is 6-6. In 25 cells with two chains, 7 were apparently regular in chromosome arrangement and 18 showed either single or double non-disjunction. The single cell observed with three chains had an apparent regular orientation of chromosomes. It is evident that non-disjunction is influenced by chromosome arrangement. In *Rhoeo*, chromosome rings show non-disjunction in about 90 percent of the figures examined, while chains show non-disjunction in only 60 percent of the figures.

#### DISCUSSION

Chromosome ring formation resulting from segmental interchange is usually associated with pollen sterility due to non-disjunction of chromosome segments. If adjacent chromosomes pass to the same pole, the resulting microspores should be sterile owing to deficiency of chromosome segments; but if adjacent chromosomes pass to opposite poles, each microspore should have a complete chromosome complement and would be capable of further development. The percentage of non-disjunction in segmental interchange rings may be very low in certain genera and high in others. A comparison of the ring-forming chromosomes in different genera should throw some light on the cause of non-disjunction in chains and rings of chromosomes.

CLELAND (1929) has found that adjacent chromosomes pass to opposite poles in about 80 percent of the rings of *Oenothera lamarckiana*. Most of the chromosomes have approximately median fiber constrictions, and the 12 chromosomes of the ring are attached end to end.

In *Datura*, BLAKESLEE (1929) finds normal pollen fertility in plants with a ring of four chromosomes. He assumes that adjacent chromosomes in the ring always pass to opposite poles, a situation resulting in fertile combinations, but no data on chromosome distribution are given. The chromosomes of *Datura* have approximately median spindle-fiber constrictions, and homologous chromosomes are united only at the ends in both rings and bivalents.

Chromosome rings and chains in *Zea* have been found by McCLINTOCK (1930), BURNHAM (1930, 1932), and by BRINK and COOPER (1931, 1932). In plants with a ring of four chromosomes, the pollen sterility is about 50 percent; with two rings, it is about 75 percent, as would be expected if adjacent chromosomes pass to the same pole or to opposite poles at ran-

dom. BURNHAM (1932) found that non-disjunctional and disjunctional segregation occur with about equal frequency. The attachment constrictions of *Zea* are more or less sub-terminal, and subterminal chiasmata are found in many of the rings.

PELLEW and SANSOME (1931) found that ring formation in *Pisum* is associated with about 50 percent pollen sterility, and in about half the cases examined, non-disjunction occurs in the ring, although no actual data on frequency of different types of segregation are presented. In the rings of four chromosomes, interstitial and subterminal chiasmata were frequently found. Two of the chromosomes appear to have sub-terminal fiber constrictions.

GAIRDNER and DARLINGTON (1932) find rings of four and six in *Campanula*. These writers state that, in the rings, the chromosomes were arranged non-disjunctionally in about 30 percent of the cells; but the published data (table 2) show 38 percent non-disjunction in rings and 65 percent in chains, or an average of 41 percent non-disjunction. The chromosomes of *Campanula* have approximately median fiber constrictions, and in both rings and bivalents the chromosomes are united only by terminal chiasmata.

Segmental interchange rings with relatively isobrachial chromosomes attached by terminal chiasmata seem to be more regular in disjunction than rings with heterobrachial chromosomes or rings with interstitial and subterminal chiasmata. The first group includes *Datura* with practically no non-disjunction, *Oenothera* with 20 to 30 percent non-disjunction, and *Campanula* with 30 to 40 percent non-disjunction. The second group includes *Zea*, *Pisum*, and *Tradescantia*, each with about 50 percent non-disjunction, and *Rhoeo* with about 80 percent non-disjunction.

In *Tradescantia edwardsiana*, the 4 chromosomes involved in the ring are apparently heterobrachial, as indicated by the position of the interstitial or subterminal chiasmata. In the non-disjunction types of chains, the short segments are oriented towards the poles, while the longer segments are frequently united by subterminal chiasmata (figures 3, 4, and 5). These configurations may be due to the greater repulsion between the long homologous segments. In *Rhoeo*, however, the adjacent long segments may pass to the same pole (SAX 1931, figures 8 and 9). But at least one of the four heterobrachial chromosomes is involved in cases of non-disjunction. Apparently the relative lengths of the chromosome segments in the ring do influence the regularity of disjunction, but the available data hardly warrant further discussion.

According to GAIRDNER and DARLINGTON (1932), chains disjoin less regularly than rings in both *Campanula* and *Rhoeo*. This conclusion will not hold for *Rhoeo* or *Tradescantia* and is of doubtful validity in *Cam-*

panula. The 13 elongated S-shaped chains in *Campanula* (table 2) are classed as non-disjunctional, but they may be regularly distributed so that adjacent chromosomes pass to opposite poles. Figures of these chromosome types in *Campanula* are not shown, and they have never been observed by the writers in either segmental interchange chains or in tetravalent chromosomes of *Tradescantia*. If these 13 chains are regular in distribution, the percentage of non-disjunction is greater in rings than in chains. In the interchange plant, *Tradescantia edwardsiana*, non-disjunction was found in all but one of the 27 rings observed, while in the 523 chains, the regular and non-disjunctional types were found in about equal proportions. Non-disjunction was found in 90 percent of the rings but in only 60 percent of the chains in *Rhoeo*. The greater regularity of disjunction in chains can be attributed to the greater flexibility in chromosome arrangement, which enables homologous segments to repel each other with greater frequency than is possible in rings.

The effects of rigidity in rings or chains are also clearly shown in the interchange complexes of *T. edwardsiana*. The chains and rings with no subterminal chiasmata were non-disjunctional in only 35 percent of the cells. With one subterminal chiasma, the percentage of non-disjunction was 55; with two subterminal chiasmata, it was 64 percent; and when there were three subterminal chiasmata in the chain or ring, all figures were non-disjunctional. It is clear that the greater rigidity of the chains or rings caused by subterminal chiasmata increases the proportion of non-disjunction.

GAIRDNER and DARLINGTON have suggested that the rigidity caused by interstitial chiasmata in *Pisum* is the cause of the frequent non-disjunction (50 percent) in the interchange rings. The data reported above demonstrated that interstitial chiasmata do have an important effect upon the percentage of non-disjunction in *Tradescantia*. But the presence of interstitial chiasmata is only one of several factors which influence the type of segregation. In *Rhoeo* with no subterminal chiasmata, non-disjunction is found in about 80 percent of the cells. In the segmental interchange *T. edwardsiana*, about 60 percent of the rings and chains have subterminal chiasmata, but non-disjunction is found in only half of the pollen mother cells. Only 40 percent of the interchange rings and chains in *T. reflexa* have subterminal chiasmata, but non-disjunction occurs in over 80 percent of the cells. The quadrivalents of *T. occidentalis* have only terminal chiasmata, and adjacent chromosomes pass to the same pole in only 20 percent of the cells. In *Rhoeo* and *T. reflexa* with high non-disjunction, the arms of certain chromosomes vary considerably in length. In *T. edwardsiana*, these differences are less extreme, and in *T. occidentalis* the chromosomes which form quadrivalents usually have approximately median constrictions.

tions. Non-disjunction is increased by ring formation, by the position of spindle fibers and relative lengths of chromosome segments in the ring or chain, and by the presence of interstitial or subterminal chiasmata.

The percentage of non-disjunction in segmental interchange plants is usually closely associated with the degree of pollen sterility. The Dexter plant, *T. reflexa*, is exceptional, since it has over 80 percent non-disjunction in the rings and chains and an average of 65 percent pollen fertility. The high degree of fertility may be due to interchange involving the translocation of all, or almost all, the one chromosome to a segment of another, so that the constitution of the four chromosomes would be Aa, aB, Bb, and bBA. Such an interchange complex would produce the very long chromosomes observed in rings and in microspore divisions, and would account for the high pollen fertility.

About 30 percent pollen sterility was found in a diploid, *T. edwardsiana*, with 6 bivalents. This plant may have unequal segmental interchange involving segments too short to pair, so that only bivalents are produced. If one translocated segment is too small to have a lethal effect, the pollen sterility would be only 25 to 30 percent. BURNHAM (1932) finds only 25 percent sterility in a case of unequal interchange in *Zea* and has suggested that only bivalents might be formed if only small segments were interchanged. The pollen sterility could also be accounted for on the assumption that one chromosome is deficient for a segment which is translocated to a non-homologous chromosome.

Segmental interchange reduces crossover frequency in *Drosophila* (DOBZHANSKY 1931), but in *Tradescantia*, chiasma frequency is about the same in interchange rings and bivalents. The average chiasma frequency is 1.7 for the four bivalents of *T. edwardsiana* and 2.9 for the four interchange chromosomes including the 98 bivalents. However, the rings and chains probably include the two "pairs" of heterobrachial chromosomes, and three of the four bivalents are isobrachial, so that the potential amount of chiasma formation may differ in the two classes. In the *T. reflexa* from Dexter, the average chiasma frequency is 1.76 for bivalents and 3.84 for rings, or a slightly higher frequency in the rings, on the basis of bivalents. The percentage of subterminal chiasmata per chromosome in chains and rings is about the same as it is in the bivalents in both species of *Tradescantia*.

In an earlier paper (SAX 1931), it was suggested that segmental interchange may result from interlocking of bivalent chromosomes, although it is known that the action of X-rays produces translocations in the mature sperm of *Drosophila* and in the pollen of *Zea*. The relative frequency of interlocked bivalents in *Oenothera*, *Campanula* and *Tradescantia* does suggest a correlation between interlocking and segmental interchange,

but it is possible that interlocking is due primarily to interchange of segments too short to cause the formation of chains or rings. The attraction between these short segments might bring the chromosomes in closer contact at the time of pairing, so that interlocking of non-homologous chromosomes would occur. In rare cases chains or rings of four chromosomes have been observed in *Tradescantia* species which usually have six bivalents. Over ten such figures have been observed in *T. edwardsiana* and *T. bracteata*, one of which is shown in figure 11. These figures suggest that segmental interchange chromosomes may originate at meiosis owing to breaks in interlocked bivalents, but it is also possible that they represent segmental interchange types of earlier origin which involve such short segments that ring and chain formation rarely occurs.

The interlocking of bivalents is of interest in relation to chiasma formation and the mechanism of crossing over. As CATCHESIDE (1931) has pointed out, interlocking of bivalents can occur only at alternate internodes if chiasmata are formed by alternate opening out of pairs of chromatids in reductional and equational planes. If chiasmata are the result of previous crossovers, then one bivalent could be locked with another at any internode. GAIRDNER and DARLINGTON (1932), in their discussion of interlocking in *Campanula*, state that "Interlocking does not seem to occur here (or in any other organism) between the chromatids of paired or unpaired chromosomes. Yet such interlocking should occur both with and without terminalization if the chromatids ever separate equationally at diplotene."

If chiasmata are caused by alternate opening out of pairs of sister and non-sister chromatids, interlocking could occur only at internode 2, as shown in figure 15 and never at internodes 1 or 3.

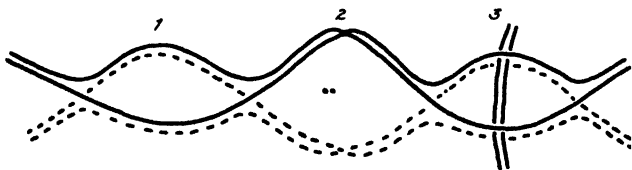


FIGURE 15.

Interlocking of one chromosome between the chromatids of a bivalent, as shown at internode 3, could not occur because the closely paired non-sister threads could move apart in an equational plane only with difficulty, owing to the interlocked pair of chromatids between them. The interlocking at pachytene would prevent a close association of non-sister threads in the region of interlocking, and a reductional opening out of paired sister chromatids would have been initiated at the earliest pachytene stage of pairing. We would expect, then, that interlocking would in-

volve only the reductional internodes and that interlocking between chromatids would never occur.

In almost all of the described cases of interlocked bivalents, only one internode is found in each pair of chromosomes, and the two chiasmata are usually terminal in each bivalent. This type of association would be expected where chromosomes have an approximately median fiber attachment point, because interlocking could occur only at the median internode, unless more than three internodes are formed, or unless three chiasmata are formed distal to the spindle fiber. Terminalization or breaks in the chiasmata could account for ring bivalents at diakinesis and metaphase.

If chiasmata are the result of previous crossovers and only sister chromatids are paired at early diplotene, as DARLINGTON and others assume, the interlocking of bivalents could occur at any internode. According to GAIRDNER and DARLINGTON, *Campanula* bivalents have from two to six chiasmata at diplotene. These chiasmata are said to be formed "interstitially and at random." CATCHESIDE (1931) found that three is the most usual number of chiasmata in *Oenothera* bivalents at diplotene, but as many as four or five may be formed. As many as four or five chiasmata have been observed in *Tradescantia* bivalents at late diplotene stages. With such a high chiasma frequency at diplotene in these genera, there should be many cases of distal interlocking of bivalents. In *Campanula*, proximal interlocking occurs in about 20 percent of the pollen mother cells while "distal interlocking seems to be much rarer." The apparent contact of rings is interpreted as distal interlocking with the distal internode terminalized to such an extent that no distal loop can be observed (GAIRDNER and DARLINGTON, figures 16 C, E, G, and J). Proximal interlocking of bivalents is frequently found in *Oenothera*, but distal interlocking is very rare and has never been clearly demonstrated (CATCHESIDE 1931). *Tradescantia* bivalents with two internodes are, in rare cases, locked with a rod bivalent, but it was impossible to determine whether or not the locked internode was proximal or distal to the spindle fiber. In all these genera the bivalents usually take the form of rings with terminal or sub-terminal chiasmata. On either theory of chiasma formation, practically all the interlocking of such ring bivalents would appear to involve proximal internodes. If all but two chiasmata pass off the ends of each bivalent by terminalization, only proximal interlocking could occur. But if terminalization simply accumulates the chiasmata at the ends of the chromosomes, as GAIRDNER and DARLINGTON assume to be the case in *Campanula*, then distal interlocking should be at least as frequent as proximal interlocking if chiasmata are the result of previous crossovers. If chiasmata are caused by alternate pairing of sister and non-sister chromatids, distal interlocking



should be rare, which is the case in all the genera with interlocked bivalents.

#### SUMMARY

Chains or rings of four chromosomes were found in two diploid plants of *Tradescantia edwardsiana*. The average chiasma frequency in the segmental interchange complex was 2.9 including potential chains, as compared with an average chiasma frequency of 1.7 for the four bivalents. The prevalence of chains and the position of subterminal chiasmata in the rings and chains are attributed to the relative lengths of the chromosome segments which pair. Non-disjunction is more frequent in rings than chains, and is more frequent in chains with one or more subterminal chiasmata than in chains with only terminal chiasmata. Non-disjunction in rings and chains was about 50 percent, which is in accord with the degree of pollen sterility found.

About 30 percent pollen sterility was found in a plant of *T. edwardsiana* with 6 bivalent chromosomes. This sterility is attributed to segmental interchange involving unequal segments, or to a translocation resulting in deficiency and duplication of chromosome segments. Interlocking of some of the bivalent chromosomes was found in almost all pollen mother cells of this plant.

In a diploid *T. reflexa* from Dexter, Michigan, a ring of four chromosomes and four bivalents was usually found at meiosis. The average chiasma frequency in the rings or chains was 3.84 and was 1.76 for the bivalents. Non-disjunction was found in 78 percent of the rings and chains, but about 60 percent of the pollen was good. This degree of fertility is attributed to a segmental interchange which is in part a duplication.

Chiasma frequency in bivalents of segmental interchange plants is about the same as in normal diploids. Segmental interchange produces only a slight reduction in chiasma frequency and does not materially affect the percentages of terminal and sub-terminal chiasmata.

An analysis of non-disjunction in the rings and chains of 12 chromosomes in *Rhoeo* shows that non-disjunction is much more frequent in rings than in chains. The total amount of non-disjunction found was 79 percent, which is in accord with the pollen sterility found.

A comparison of the amount of non-disjunction reported in *Oenothera*, *Datura*, *Campanula*, *Zea*, *Pisum*, and in *Tradescantia* and *Rhoeo*, shows that the percentage of non-disjunction is dependent, in part, at least, on the proportional lengths of homologous segments in the chromosomes of the segmental interchange complex and the proportion of interstitial or subterminal chiasmata. Greatest regularity in disjunction of rings or chains is found where the chromosomes are isobrachial and are attached only by terminal chiasmata.

Interlocked bivalents are frequently found in *Tradescantia* and in other genera in which segmental interchange rings are found. Breaks and a re-union of non-homologous segments in such interlocked bivalents might account for the segmental interchange rings. Chains or rings of four chromosomes are occasionally found in *Tradescantia* plants which normally have only six bivalents. The prevalence of interlocked bivalents in genera where segmental interchange is found may, however, be due to interchange of chromosome segments too short to insure pairing in chains or rings, but long enough to cause some interlocking of chromosomes at the time of pairing.

The prevalence of proximal interlocking of non-homologous chromosomes in *Oenothera*, *Tradescantia*, and *Campanula*, is in accord with the assumption that chiasmata are the result of alternate pairing of sister and non-sister chromatids.

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# NINE INDEPENDENTLY INHERITED AUTOSOMAL FACTORS IN THE DOMESTIC FOWL<sup>1</sup>

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## CHROMOSOMAL COMPLEX

The question of the exact number of chromosomes in the cells of the domestic fowl, *Gallus domesticus*, still remains an unanswered one. As cytological material the cells of this species have proved to be unsatisfactory. HANCE (1926) who has given considerable time to the cytology of the domestic fowl states that much of the difficulty with this form has been due to the failure of the fixation chemicals to penetrate the tissue.

Most of the chromosomes of the fowl are large enough to be easily recognized but a few are very small. In these very small chromosomes is the source of the difference of opinion as to the exact count. STEVENS (see BORING 1923) gave the unreduced number as 34, SHIWAGO (1924) placed it at 32 and HANCE (1924) obtained a count of 34 or 35. All workers have recognized the sex chromosome which is the largest member of the complex. This chromosome is paired in the male but there remains a difference of opinion as to whether it is unpaired or has an unlike mate in the female.

Considerable attention (SEREBROVSKY and WASSINA, 1926), (WARREN 1928), (HERTWIG and RITTERHAUS 1929) has been given to the mapping of the factors of the sex chromosome since they are readily recognized by their crisscross type of inheritance. However, very little study has been made of the autosomal factors. Regardless of the fact that there remains a question as to the exact number of autosomes in the fowl the problem of linkage relations justifies some consideration. The only well established case of linkage between autosomal factors is that for rose comb and creeper (SEREBROVSKY and PETROV 1928). The large number of known workable characters in the fowl offer material for study of the linkage relations in this species.

## CHARACTERS CONSIDERED

The writer has selected nine fairly well known characters of which the contrasted expressions were for the most part recognizable in the day-old chick. This latter condition is especially desirable since there is avoided the losses from early mortality and the expense of carrying the birds to maturity. Some of the characters have been included not because of their adaptability for linkage studies but because they are part of the make-up of the breeds used.

<sup>1</sup> Contribution No. 67 from the Department of Poultry Husbandry.

The dominant members of the allelomorphic pairs were, naked-neck, rumpless, white skin, leg-feathering, rose comb, pea comb, crest, polydactyly, and dominant white. With the exception of crest and white skin these characters were all recognizable in the day-old chick. Much of the data were obtained from the examination of embryos taken from the shell

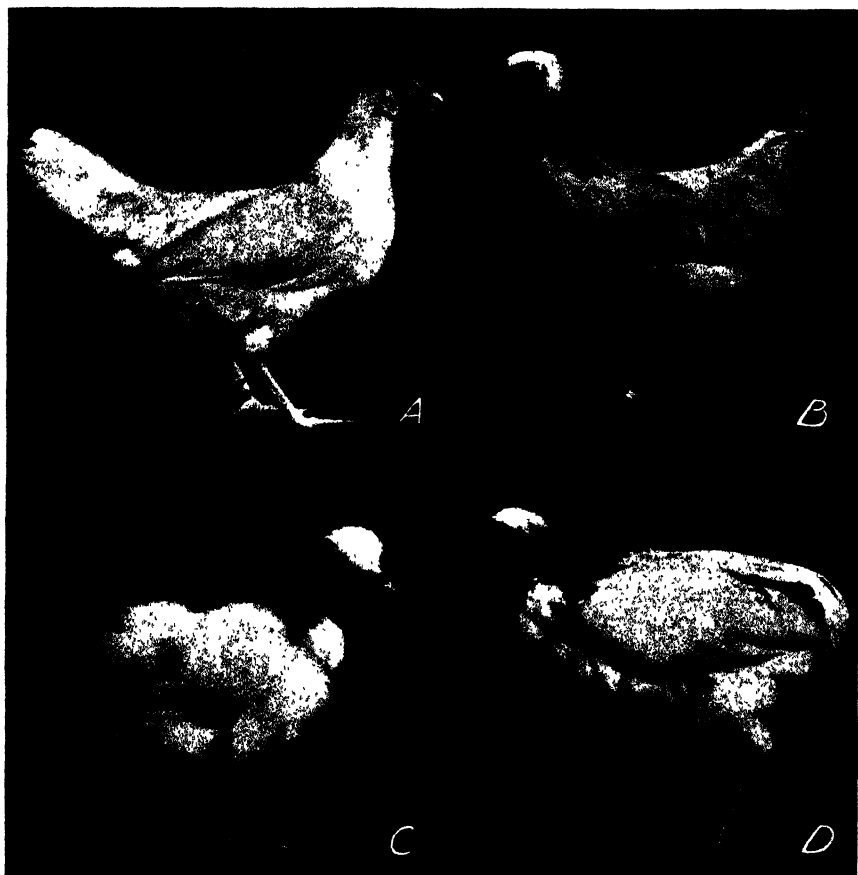


FIGURE 1.—The above reproduced birds show eight of the characters considered in this paper as follows: Naked-neck, C and D; rumplessness, C and D; leg-feathering, B and C; rose comb, D; pea comb, B; polydactyly, C; crest, C; and dominant white, A. Differences in skin color could not be shown by means of photographs.

on the eighteenth day of incubation since this eliminated much of the work involved in individual pedigreeing of the chicks. In cases where the characters could not be recognized at hatching the individuals were described at eight and twenty-four weeks of age.

#### NAKED-NECK

The naked-neck individuals are characterized by the complete absence of feathers on the neck. There is also a tendency to slightly restrict the

feather tracts in other regions of the body. Individuals exhibiting this character show excellent viability and it is expressed very early in embryonic development. The ease of classification makes it a very excellent character for genetic studies. This character has been shown by DAVENPORT (1914) and CREW (1922) to behave as a simple dominant. Table 1 presents the results of backcrossing  $F_1$  birds to the recessive normal condition. This table includes results both from chicks and adults. Most of those included in the chick classification were recorded on the eighteenth day of incubation. This section of the table also includes a few chicks that were described at the day-old stage but died soon after. In case the character is of low viability the chick section of the table should give better ratios than the adult section since it includes individuals which do not have sufficient vigor to escape from the shell and thus would be eliminated from the adult classification.

TABLE 1

*Backcross of males and females heterozygous for naked-neck to recessive normals.*

MALE NUMBER	HETERO- ZYGOUS SEX	ADULT DESCRIPTION				CHICK DESCRIPTION			
		FEMALES		MALES		FEMALES		MALES	
		NAKED	NORMAL	NAKED	NORMAL	NAKED	NORMAL	NAKED	NORMAL
1061M	♂	38	39	57	38	32	28	38	35
1074M	♂	34	36	41	41	47	38	53	52
1099M	♂	47	55	69	72	102	61	76	92
1102M	♂	25	31	30	31	30	16	17	9
1143M	♂	.	.	.	.	25	28	39	38
1144M	♂	59	48	48	46	22	20	23	28
1145M	♂	.	.	.	.	59	58	52	65
1147M	♂	16	23	22	26	.	..	..	..
1148M	♀	60	72	43	48	.	.	.	..
1149M	♂	..	..	..	..	51	44	49	47
1150M	♂	21	27	27	24	..	..	..	..
1159M	♀	..	.	.	..	18	11	18	14
Total		300	331	337	326	386	304	365	380

Grand Total—1388 Naked to 1341 Normals

The results show no sexual dimorphism in the expression of the character. The totals of the adult classification approach very closely to the expected 1 to 1 ratio with 637 naked to 657 normals. The chicks showed a preponderance of naked, 751 to 684 normals. The grand total gave 1388 naked to 1341 normals.

### *Rumplessness*

The character is a well known dominant in poultry. DUNN (1925) found that this condition appears occasionally in a non-heritable form in chickens. Genetically it behaves as a simple dominant. The primary morphological

change in the rumpless fowl is the absence of the five free caudal vertebrae and the pygostyle. The loss of this portion of the skeleton causes the oil gland and most of the larger tail feathers to be absent.

Table 2 gives the results of backcrossing individuals heterozygous for rumpless to normals. As is the case of all tables in this publication, the chick section of the table includes individuals that were removed from the shell on the eighteenth day of incubation. The very close approximation of a 1 to 1 ratio in both the chick and adult sections of the table indicates that this character in no way handicaps the chick during development. The totals for the combined results were 798 rumpless to 833 normals. The rumpless condition may be recognized by touch as early as the eighteenth day of incubation.

TABLE 2

*Backcross of males and females heterozygous for rumplessness to recessive normals.*

MALE NUMBER	HETERO- ZYGOUS SEX	ADULT DESCRIPTION				CHICK DESCRIPTION			
		FEMALES		MALES		FEMALES		MALES	
		RUMPLESS	NORMAL	RUMPLESS	NORMAL	RUMPLESS	NORMAL	RUMPLESS	NORMAL
1061M	♂	36	41	56	39	33	27	39	34
1074M	♂	30	40	38	44	55	30	51	54
1102M	♂	25	31	19	42	27	19	11	15
1143M	♂			..		24	29	37	40
1144M	♂	43	64	49	45	21	21	24	27
1147M	♂	15	24	24	24	.	.	..	..
1148M	♀	67	65	50	41				..
1159M	♀	..				11	18	13	19
Total		216	265	236	235	171	144	175	189

Grand Total—798 Rumpless to 833 Normals

### *Skin color*

The white and yellow skin colors characteristic of various breeds of poultry constitute an allelomorphic pair of autosomal characters. White skin has been found to be dominant to the yellow. Since the difference is usually more easily detected in the shank the factor is sometimes referred to as one influencing shank color rather than skin color. Black pigmentation found in the shanks of some breeds interferes with classification with respect to the skin color factor. An examination of the bottom of the foot of birds carrying the dark shanks will usually make it possible to determine its skin color. Skin color cannot be accurately determined until the chick is beyond a month of age. The xanthophyll pigment which gives the shank its yellow color fades in heavy producing females and is not well developed in chicks reared on feeds deficient in vitamin A. Errors of classification from these sources are not great and the ideal age at which classi-

fication may be made is just before the bird reaches sexual maturity. LAMBERT and KNOX (1927), DUNN (1925) and PUNNETT (1922) have published material on the inheritance of this character.

In table 3 are presented the data upon the segregation of skin color factors. Since classification cannot be made at hatching the chick section of the table has been omitted. The totals of 481 white skin to 477 yellow give a very close approach to a 1 to 1 ratio.

TABLE 3  
*Backcross of males and females heterozygous for white skin to recessive yellow skin.*

MALE NUMBER	HETEROZYGOUS SEX	ADULT DESCRIPTION			
		WHITE	FEMALES YELLOW	MALES WHITE	YELLOW
1061M	♂	28	26	21	37
1074M	♂	38	32	41	41
1099M	♂	50	52	86	55
1144M	♂	50	41	35	40
1146M	♂	29	32	29	40
1147M	♂	12	8	14	22
1150M	♂	25	23	23	28
Total		232	214	249	263

Grand Total—481 White Skin to 477 Yellow Skin

### *Leg-feathering*

Some of the most comprehensive studies of this character are by PUNNETT and BAILEY (1918), SEREBROVSKY (1926), DUNN and JULL (1927) and LAMBERT and KNOX (1929). There is rather general agreement among them that the more sparse feathering of the legs found in the Langshan is due to a single factor difference while the heavy leg-feathering found in the Cochins and other Asiatic breeds is due to two pairs of factors. The two factors are dominant and cumulative in effect. This character is fully expressed in the day-old chick. Slight degrees of feathering may be more easily detected in the newly-hatched chick since the feathers may be lost by accident during growth.

The data in table 4 are based upon leg-feathering as it is found in the Light Brahma and Silky Bantam breeds. In these two breeds the leg-feathering is of the heavy type. The results are from backcrossing heterozygous individuals to non-feathered mates. The second column indicates the sex of the heterozygote. Males 1041M, 1061M, and 1149M were smooth-legged birds and mated to heterozygous feathered females which were the offspring of a Light Brahma male. Male 1079M was heterozygous for the Light Brahma type of feathering. Males 1144M and 1147M were heterozygous for the Silky Bantam leg-feathering. Males 1099M, 1101M, and

TABLE 4

*Backcross of males and females heterozygous for leg-feathering to recessive normals.*

MALE NUMBER	HETERO- ZYGOUS SEX	ADULT DESCRIPTION				CHICK DESCRIPTION			
		FEMALES		MALES		FEMALES		MALES	
		FEATHERED	NORMAL	FEATHERED	NORMAL	FEATHERED	NORMAL	FEATHERED	NORMAL
1041M	♀	64	79	79	76	..	.	..	..
1061M	♀	33	36	48	42	29	31	39	34
1079M	♂					49	41	40	32
1099M	♂	53	49	64	77	84	79	82	86
1101M	♀		.			31	67	26	55
1102M	♂	19	37	13	48	13	33	4	22
1144M	♂	47	60	53	41	15	27	14	37
1147M	♂	15	24	14	34		.	.	..
1149M	♀				.	18	77	33	63
Total		231	285	271	318	239	355	238	329

Grand Total—979 Feathered to 1287 Normals

1102M were all heterozygous  $F_2$  segregates originating from the Light Brahma.

An examination of table 4 shows that in all matings the number of feathered-legged individuals was only equal to or less than that for smooth-legged ones. This would indicate that our material carried only a single factor for leg-feathering since on a cumulative two-factor basis it would be expected that the number of feathered would exceed the smooth.

*Rose comb*

This well known character requires no extensive description since it was one of the characters earliest studied in poultry. At hatching it is easily distinguished from its allelomorph, single comb.

BATESON early found that rose comb behaved as a simple dominant toward single. Results in table 5 showing the offspring from a backcross of

TABLE 5

*Backcross of males and females heterozygous for rose comb to recessive single comb individuals.*

MALE NUMBER	HETERO- ZYGOUS SEX	ADULT DESCRIPTION				CHICK DESCRIPTION			
		FEMALES		MALES		FEMALES		MALES	
		ROSE	SINGLE	ROSE	SINGLE	ROSE	SINGLE	ROSE	SINGLE
1074M	♂	37	33	37	45	44	41	58	47
1079M	♂	..	..	..	..	45	45	42	30
1101M	♀	..	..	..	..	41	57	43	38
1144M	♂	56	51	46	48	22	20	25	26
Total		93	84	83	93	152	163	168	141

Grand Total—496 Rose to 481 Single.



heterozygous individuals to those having single combs are in agreement with this view. The very close approximation of a 1 to 1 ratio indicates the good viability of the character. The similarity of results in the adult and chick section of the table shows that the character may be accurately recognized at hatching.

### *Pea comb*

This character was also early studied. This gene in combination with that for rose produces a distinct type of comb called walnut. The fact that the double dominant condition may be recognized has made the cross of rose by pea a classic experiment as an example of a di-hybrid cross. Pea comb has always been listed as dominant to single comb and the dominance is practically perfect when the single comb breed is a small combed variety as is true in most heavy breeds. However, in crosses with the larger combed Mediterranean breeds the adult condition of the comb is much more like a single than a pea. The comb of the heterozygous bird is usually of a lopping single type carrying ridges on the side. There is seldom any difficulty in recognizing the heterozygote but the comb here is more like that of the single parent than that with the pea comb.

In table 6 the grand total shows a slight shortage of pea combs since the ratio is 920 pea to 1037 single. The totals for the two sections show that the shortage is all accounted for in the chick section of the table. The adult section totals 406 peas to 411 singles while the totals for the chick section are 514 to 626 singles. There is some variation in the combs of chicks heterozygous for pea and in some cases they might at that time be classed as singles. The genes for pea comb used in these studies were derived from the Light Brahma breed.

TABLE 6  
*Backcross of males and females heterozygous for pea comb to single comb individuals.*

MALE NUMBER	HETERO- ZYGOUS SEX	ADULT DESCRIPTION				CHICK DESCRIPTION			
		FEMALES		MALES		FEMALES		MALES	
		PEA	SINGLE	PEA	SINGLE	PEA	SINGLE	PEA	SINGLE
1041M	♀	66	77	67	88	..	..	..	..
1061M	♀	36	33	42	48	..	..	..	..
1099M	♂	65	37	69	72	76	87	83	85
1101M	♀	..	..	..	..	26	36	24	29
1102M	♂	30	26	31	30	19	27	10	16
1143M	♂	..	..	..	..	25	28	36	38
1145M	♂	..	..	..	..	52	65	55	61
1149M	♀	..	..	..	..	38	57	39	57
1159M	♀	..	..	..	..	18	22	13	18
Total		197	173	209	238	254	322	260	304

Grand Total—920 Pea to 1037 Single

*Crest*

The crest characterized by elongated and more erect feathers on the head was derived from the Silky Bantam. The various workers (BATESON and SAUNDERS 1902, DAVENPORT 1909, BARFURTH 1911, DUNN and JULL 1927), have all placed this character on a monofactorial basis. The data here are rather limited but as shown in table 7 substantiate this view. The totals of 108 crested to 124 normals show a slight deficiency in the crested class.

TABLE 7  
*Backcross of heterozygous crested males to normal females.*

MALE NUMBER	HETEROZYGOUS SEX	ADULT DESCRIPTION			
		FEMALES		MALES	
		CREST	NORMAL	CREST	NORMAL
1144M	♂	48	46	35	42
1147M	♂	10	13	15	23
Total		58	59	50	65

Grand Total—108 Crested to 124 Normals

*Polydactyly*

Although considerable study has been given this character its mode of inheritance has not been completely analyzed. Investigators (DUNN and JULL 1927, PUNNETT and PEASE 1929) have agreed that it is probably on a simple monofactorial basis. PUNNETT and PEASE have suggested that an inhibitor may interfere with its regular segregation since four-toed individuals sometimes transmit the five-toed condition. The extra-toed condition has been found to be dominant to the more usual four-toes. Polydactyly as here considered was introduced through the Silky Bantam.

The results in table 8 show a large shortage of polydactyls. The totals are 167 polydactyls to 259 normals. On a monofactorial basis equal num-

TABLE 8  
*Backcross of males and females heterozygous for polydactyly to normal individuals.*

MALE NUMBER	HETERO- ZYGOUS SEX	ADULT DESCRIPTION				CHICK DESCRIPTION			
		FEMALES		MALES		FEMALES		MALES	
		POLY- DACTYLY	NORMAL	POLY- DACTYLY	NORMAL	POLY- DACTYLY	NORMAL	POLY- DACTYLY	NORMAL
1144M	♂	46	61	33	60	19	23	19	32
1147M	♀	11	28	19	30	..	..	..	..
1149M	♀	..	..	..	..	9	14	11	11
Total		57	89	52	90	28	37	30	43

Grand Total—167 Polydactyls to 259 Normals

bers would have been expected. The preponderance of normals occurs in each of the three matings.

### *Dominant white*

Dominant white plumage which is characteristic of the White Leghorn breed is a well known color factor in the fowl. Its dominance to color is incomplete since some black flecks or dinginess usually occur; however, the heterozygous individual is always predominantly white.

The totals given in table 9 show a slight preponderance of whites, there being 674 whites to 627 coloreds.

TABLE 9

*Backcross of males and females heterozygous for dominant white to colored individuals.*

MALE NUMBER	HETERO- ZYGOUS SEX	ADULT DESCRIPTION				CHICK DESCRIPTION			
		FEMALES		MALES		FEMALES		MALES	
		WHITE	COLOR	WHITE	COLOR	WHITE	COLOR	WHITE	COLOR
1101M	♀	..	..	..		87	73	60	74
1143M	♂	.	..	.	..	31	22	41	33
1145M	♂	..	..	.		72	45	72	45
1146M	♂	35	26	31	38	.		.	
1150M	♂	30	24	29	32	.		.	
1159M	♀	..	..	..	..	38	33	27	38
1181M	♂	.		.		22	38	18	28
1184M	♂	..	.	.		37	42	44	36
Total		65	50	60	70	287	253	262	254

Grand Total—674 White to 627 Colored

### TESTS FOR ASSOCIATION OF CHARACTERS

The results on tests for linkage relations were obtained from crosses of birds carrying in varying combinations the characters considered. Usually three or more were considered at a time but for the sake of clarity in presentation the tabulation has been in pairs. In as much as the purebred females usually produce better than the crossbreds, the backcrossed heterozygote has usually been a male. An additional advantage of making the cross in this direction is that in instances where it was difficult to obtain combinations desired, a single multiple heterozygote of the male sex when obtained would give as many offspring as several such females. In cases where the female has been the heterozygote it has been indicated in the table.

All data presented are from backcrosses of multiple heterozygotes to multiple recessives. Both chick and adult descriptions were used. The chick descriptions apply to day-old chicks and embryos taken from the shell on

the eighteenth day of incubation. Skin color and crest could not be recognized in day-old chicks. Both repulsion and coupling were employed for testing for linkage. There remains some question as to the number of factors involved in the inheritance of leg-feathering and polydactyly. If more than one pair of factors determine the expression of the character the linkage reactions will be affected. However, if one of the genes involved were at all closely associated with a factor for which a test is being made, the linkage should be detectable.

In making tests for linkage one is immediately confronted with the question of what should be considered as a minimum number of individuals necessary for establishing the fact of the existence of independent assortment. In a survey of this nature it is desirable to confine the numbers to the dependable minimum in order to cover as much ground as possible.

At the initial stages of a study of this kind we can probably say that cases of crossing over of 40 percent or more are of little value as well as very difficult to definitely establish. Such loose linkages are of little use in mapping the genes of the chromosomes and can probably be studied to an advantage only when linkage groups of the fowl are better known.

By use of the formula  $0.6745 \sqrt{p \cdot q / n}$  for calculation of the probable error, it is found for cases of crossing over of less than 40 percent, a total of 182 individuals is sufficient for establishing the fact that the deviation is not due to chance. This calculation is based upon the assumption that a deviation of 4 times its probable error is significant. The numbers required increase very rapidly as the percentage of crossing over increases; for example, crossing over of 45 percent requires 730 individuals to be assured that the deviations are not due to chance. Since the detection of linkages where the crossing over percentage is less than 40 is desired we have considered the total of 200 as sufficient for the establishment of the fact of independent assortment. In most cases our numbers are considerably beyond the value suggested.

#### PREVIOUSLY REPORTED LINKAGES

There seems to be only one well supported case of autosomal linkage in the fowl. The linkage of rose comb and creeper was reported by SEREBROVSKY and PETROV (1928). Creeper is characterized by a shortening of the leg bones. Their preliminary results indicated that there was approximately 8 percent of crossing over between these two factors. Later results by these same authors (1930) placed the percentage of crossing over at 10. However, the more extensive data of LANDAUER (1931) indicate that there is less than 1 percent of crossing over between creeper and rose.

The second case of linkage in the fowl was reported by DUNN and JULL (1927) and later by DUNN and LANDAUER (1930). They found evidence for

linkage of dominant white and cerebral hernia. The difficulty of establishing this case lies in the irregular genetic behavior of cerebral hernia. They tentatively placed dominant white and cerebral hernia 2 or 3 units apart on the same chromosome.

DUNN and JULL (1927) also found some evidence for the linkage of polydactyly and dominant white. Their data were very limited, however, and the writer's own data (table 10) showed no evidence for any association of these two factors. DUNN and JULL also suggested the linkage of crest and rose comb. SEREBROVSKY and PETROV (1930) place these two factors in the same chromosome. However, the more critical results of JULL (1930) failed to substantiate the earlier findings.

SEREBROVSKY and PETROV (1930) placed creeper, rose comb, dominant white and crest all in the same linkage group. This grouping seems to be as yet rather poorly supported since SEREBROVSKY himself found no evidence for the linkage of dominant white and rose and only evidence of very loose linkage of creeper and dominant white. JULL's failure to find evidence for linkage of crest and rose removes another from the group.

SEREBROVSKY and PETROV also placed blue and naked-neck in the same linkage group with 41.7 percent of crossing over between them. His numbers, 252 non-crossovers to 173 crossovers, were large enough so that the difference between the two classes may be taken as indicative of linkage. However, the writer has data upon this same relationship which fail to agree with those of SEREBROVSKY and PETROV. My own totals were 190 non-crossovers to 204 crossovers and here the difference is not significant. SEREBROVSKY and PETROV have also reported linkage between polydactyly and split comb with 33 percent of crossing over.

#### LINKAGE RESULTS

Table 10 presents the condensed results of the linkage studies.<sup>1</sup> In this table are given only the totals in the non-crossover and the crossover classes. These sums are separated by a dash, the non-crossovers being followed by the crossovers. By following down, and from left to right, through the table one may locate each combination tested. In three combinations where our own data were incomplete, the results of SEREBROVSKY and PETROV have been included. These results are marked with an asterisk.

The probable errors have not been given but in no case is there sufficient deviation from a 1 to 1 ratio to be indicative of linkage. Table 11 was arranged in order that the significance of the deviations could be checked upon by observation. The values here were arrived at by calculating the

<sup>1</sup> Detailed tables showing age at classification of the characters, the sex in which the test for crossing over was made, and whether the test was for coupling or repulsion are on file at the BROOKLYN BOTANIC GARDEN and may be obtained for examination on request.

TABLE 10

*List of character combinations tested for linkage. The first number in each space is the total of non-crossovers and the other the total of crossovers.*

	DOMINANT WHITE	POLY- DACTILY	CREST	PEA COMB	ROSE COMB	LEG- FEATHERING	WHITE SKIN	RUMPLESS
Naked Neck	304-308	187-239	125-107	664-721	348-374	656-679	398-430	890-827
Rumpless	194-193	199-181	112-122	165-186	353-369	280-292	250-236	..
White Skin	161-148	112-110	107-115	120-123	163-155	217-248		.
Leg-feathering	157-137	199-182	115-119	727-697	325-310	.		..
Rose Comb	194-183	148-146	80- 92	388-506*	.	..		
Pea Comb	285-262	152-160*	177-173*	.		.	.	.
Crest	?	121-113	.		..	.	..	.
Polydactyly	161-149	.			.	..	.	..

\* From paper by SEREBROVSKY and PETROV.

TABLE 11

*Maximum non-significant deviations from a 1 to 1 ratio expressed in difference between the non-crossover and crossover classes.*

N-VALUE	DIFFERENCE	N-VALUE	DIFFERENCE	N-VALUE	DIFFERENCE
150	33	450	57	750	74
200	38	500	60	800	76
250	43	550	63	850	79
300	47	600	66	900	81
350	50	650	69	950	83
400	54	700	71	1000	86

maximum deviation due to chance with varying values of N. The maximum deviation was arrived at by multiplying the probable error by four, thus considering deviations greater than four times the probable error to be outside the range of chance. In order to make the values apply more readily to the data as ordinarily presented, the maximum deviation was doubled so as to express the permissible difference between the non-crossover and the crossover classes instead of the deviation from the mean. Thus in instances where the total number of individuals involved is 350, the non-crossover class may exceed the crossover class as much as 50 and not be indicative of linkage. Where the total is 400 the difference may be as great as 54. Where the *n*-value falls between those two, the significant difference may be estimated. Approximations are permitted here since it is a matter of opinion as to exactly how many times the actual difference must exceed its probable error of the difference.

Table 10 summarizes the results of 35 relationships, three of which (those marked with an asterisk) were taken from the data of SEREBROVSKY and PETROV (1930). It will be noted that the difference between the crossover and the non-crossover classes is significant in the case of rose and pea comb

(Serebrovsky and Petrov 1930). However, it is the crossovers that exceed the non-crossovers. This case has been so well established as one of independent assortment that the difference must be due to errors in classification. These three relationships taken from the work of Serebrovsky and Petrov were not considered in the writer's investigations. For the nine characters considered, this leaves only one relationship to be tested, crest to dominant white. Accidental loss of stock prepared for this particular study necessitated the postponement of the test of this relationship. Unless the single remaining relationship upon test shows linkage, it may be said that these nine characters show independent assortment. Such behavior is usually taken to indicate that each character has its gene located on a separate chromosome. In other words, each character may be taken as a marker for an independent linkage group. Although the exact number of autosomes in the fowl remains in question, it seems probable that it does not exceed 17 pairs. With only 17 autosomal linkage groups, it is not probable that the first 9 characters chosen at random and completely tested should prove each to be on a separate chromosome. The laws of chance should have given at least two linkages in the 35 tests made. The shortage is even much greater if we consider the number of reported linkages and the total number of tests that have been made.

The sex-linked group of factors in the fowl has been established by the exceptional mode of inheritance of its members rather than through their association. Due to the lack of linkage, several of the members of this group would have been placed in separate linkage groups had they not been identified by means of their mode of inheritance. Loose linkages, or long map distances, may therefore be characteristic of the fowl and account for the shortage of recognized linkage groups. When intervening genes are located, some of the pairs of characters which here have failed to show linkage may later be found to have their genes in the same chromosome. We, therefore, can only state that the nine characters studied all showed independent segregation. Since all possible tests have been made, they may tentatively be considered as markers for independent linkage groups and form a basis for continued studies in this field.

#### SUMMARY

1. The nine characters naked-neck, rumplessness, white skin, leg-feathering, rose comb, pea comb, crest, polydactyly, and dominant white show independent inheritance. (This excepts the relationship, crest to dominant white, which has not been tested.)

2. Although no linkage was found it seems improbable that each of the above characters belongs to a separate linkage group. The linkage may have been so loose that it was not detectable.

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## GENETICS OF THE FOWL. II.

### A FOUR-GENE AUTOSOMAL LINKAGE GROUP<sup>1</sup>

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While the study of sex-linked genes in the fowl has proceeded in a very satisfactory manner, geneticists have experienced some difficulty in discovering linkage groups among the autosomes of that species. In this paper there are reported certain data on a case of autosomal linkage which, when considered along with the findings of other workers, permit the definite assignment of four genes to one linkage group and give some idea of their arrangement in the chromosome.

The literature on autosomal linkage in the fowl is so scanty that it may be briefly reviewed. A start in this direction was made when DUNN and JULL (1927) reported some evidence for the linkage of dominant white, cerebral hernia and polydactyly. Additional data presented by DUNN and LANDAUER (1930) made it clear that there is close linkage between the genes for the first two of these characters and suggested that polydactyly might be in the same chromosome but comparatively loosely linked with the other two. More extensive data obtained by WARREN (1932) show independent segregation of dominant white and polydactyly in a backcross progeny of 310 fowls.

Meanwhile SEREBROVSKY and PETROV (1928, 1930) reported linkage of creeper and rose comb, with crossing over of 9.1 percent in 297 progeny from diheterozygous males. A much closer linkage of these two genes was decisively demonstrated by LANDAUER (1931) who found only 0.45 percent of crossing over between them in 4483 backcross progeny.

SEREBROVSKY and PETROV (1930) postulated that creeper also belonged in the linkage group containing dominant white, but their evidence for such an assumption was hardly adequate and the possibility is disproved by the evidence (see table 6) for the independence of dominant white and rose comb. These investigators also present evidence that the genes for blue plumage color and bare neck may be linked.

More recently SUTTLE and SIPE (1932) have reported the finding of about 28 percent of crossing over between frizzling and crest. The data presented below show that frizzling is linked with dominant white. It therefore follows that dominant white, cerebral hernia, frizzling and crest are in one linkage group.

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It must be pointed out that while a considerable number of linkage trials not reviewed above have shown only the independence of the genes tested, the possibility remains that certain pairs might be in one chromosome but too far apart to show any linkage. Thus WARREN (1932) has presented linkage-test data which, taken with some from the work of SEREBROVSKY and PETROV (1930), cover 35 of the 36 possible combinations in pairs of the following nine autosomal characters: naked neck, rumpless, white skin, dominant white, leg feathering, rose comb, pea comb, crest and polydactyly. All of these 35 trials showed independence. Since the fowl has (probably) 17 pairs of autosomes, one would expect to find by chance two cases of linkage in 35 pairs tested, and the failure to do so does not mean that certain of the pairs of characters may not subsequently be found to belong in one linkage group.

## SYMBOLS

The following symbols will be used to designate the pairs of allelomorphs considered in this paper:

<i>I</i> —dominant white	<i>i</i> —no inhibitor (of melanin)
<i>h<sub>r</sub></i> —cerebral hernia	<i>H<sub>e</sub></i> —normal skull
<i>F</i> —frizzled plumage	<i>f</i> —normal plumage
<i>C<sub>r</sub></i> — crest	<i>c<sub>r</sub></i> —no crest
<i>W</i> —white shanks	<i>w</i> —yellow shanks
<i>R</i> —rose comb	<i>r</i> —single comb
<i>C</i> —color (melanin)	<i>c</i> —recessive white

CROSSING OVER BETWEEN *F* AND *I**In females. Backcross. Repulsion phase*

It has long been known that the White Leghorn is a potentially colored bird in which pigmentation is inhibited by the dominant gene *I*. Fowls heterozygous for *I* are white but usually have a few feathers wholly or partially black. Frizzling has been shown by LANDAUER and DUNN (1930) and HUTT (1930) to be a unifactorial character dependent upon the dominant gene *F*. It is quite useful for linkage studies partly because heterozygotes are quite easily distinguished from both homozygotes and normal fowls, but also because the character is usually recognizable at two weeks or earlier.

In 1930, twelve females derived from a cross of colored frizzled females and White Leghorn male (*ff II CC*) were backcrossed to a White Orpington male. These twelve were thus of the constitution  $\frac{Fi}{fI} CC$  and the Orping-

ton was  $\frac{fi}{fi}$  cc. Since the females were homozygous for *C*, the recessive white introduced by the Orpington could not in any way complicate the 1:1 ratio of white to colored fowls expected from the segregation of *I* and *i*. Classifications were made at three weeks and in most cases were checked at four months. Both of the dominant characters are so distinct from their recessive allelomorphs that errors in classification are most unlikely. Red color was introduced by the twelve females, the dams of which were phenotypically frizzled Rhode Island Reds. Although this red is only partially inhibited by *I*, the presence or absence of the latter gene is easily recognized by the absence or presence, respectively, of black pigment in the wings and tail.

In the segregation of *F* and *I* in each of these twelve progenies (table 1) there is evident a distinct departure from the 1:1:1:1 ratio to be expected if the two genes were independent.

TABLE 1  
*Segregation of F and I in backcross, repulsion phase.*

FEMALE	FRIZZLED		NORMAL	
	WHITE <i>FI</i>	COLORED <i>Fi</i>	WHITE <i>fi</i>	COLORED <i>fi</i>
K 451	3	6	10	3
K 452	2	10	3	1
K 453	1	7	3	1
K 454	3	5	7	0
K 455	0	5	4	1
K 458	0	4	6	0
K 459	1	4	2	1
K 460	1	6	6	1
K 461	3	5	9	2
K 462	0	3	3	1
K 463	0	4	1	0
K 475	2	2	5	2
Unpedigreed eggs*	2	2	4	0
Totals	18	63	63	13

\* Included because all females in the pen were of the same genotype.

While there are 81 frizzled to 76 normal and 81 white to 76 colored, a close fit to the expected 1:1 ratio in each pair, there is a marked excess in the *Fi* and *fi* classes with a corresponding deficiency in the *FI* and *fi* groups. Since *F* and *I* entered the cross in the repulsion phase, the segregation observed is to be expected if these two genes are linked. The birds in all four classes may be grouped as follows:

Parental combinations ( <i>Fi</i> and <i>fi</i> ):	126
New combinations ( <i>FI</i> and <i>fi</i> ):	31

Crossing over in this case is thus 19.74 percent.

*In females. Backcross. Coupling phase*

The only data available for this type of cross are those obtained from a mating made in 1932 of females having the genetic constitution  $\frac{FI}{fi}$  to an Ancona male having the genotype  $ffii$ . Unfortunately only three females produced viable chicks so that the data are quite meagre. Classifications were made at two weeks and doubtful cases were re-examined at later periods. The numbers in the four possible classes were as follows:

Frizzled white	Frizzled colored	Normal white	Normal colored
$FI$	$Fi$	$fl$	$fi$
15	2	4	12
Parental combinations, $FI$ and $fi$ : 27			
New combinations, $Fi$ and $fI$ : 6			

Considering that the numbers are small, the amount of crossing over—18.18 percent—is remarkably close to that found with larger numbers in the repulsion phase.

*In the male. Backcross. Coupling phase*

In 1932, A 1064, a white male proved to be heterozygous for frizzling and for dominant white, was backcrossed to twelve colored females having normal plumage. Subsequent segregation of  $F$  and  $I$  showed that these two genes were carried by the male in the coupling phase, so that the male was  $\frac{FI}{fi}$  and the females  $ffii$ . The progeny were distributed as follows:

Frizzled white	Frizzled colored	Normal white	Normal colored
$FI$	$Fi$	$fl$	$fi$
39	5	8	30
Parental combinations ( $FI$ and $fi$ ): 69			
New combinations ( $Fi$ and $fI$ ) : 13			

The amount of crossing over in the gametes of this male was thus 15.85 percent.

Other evidence of linkage of  $F$  and  $I$  was obtained in 1931 from the mating of L 15, a male heterozygous for frizzling, dominant white and color, to five colored females which were heterozygous for frizzling and color. All of these birds were from dams  $FfIiCC$  in constitution and were sired

by a White Orpington. L 15's genetic formula was therefore  $\frac{FI}{fi} Cc$  and the five females were  $\frac{Fi}{fi} Cc$ .

The occurrence of recessive whites ( $cc$ ) among the progeny of this cross

would at first sight appear to complicate any reading of the segregation of the *I* and *i* genes, but the theoretical expectations can be easily calculated.

The data are not suitable for the computation of the amount of crossing over, but the observed distribution can be compared with that expected on the basis of the linkage found in other matings. Although crossing over between *F* and *I* in females was 19.74 and 18.18 percent in repulsion and coupling phases, respectively, there is a possibility that linkage may be stronger in males than in females, and accordingly it would seem safer to assume for L 15 an amount of crossing over approximately the same as that found in the gametes of ♂A1064, namely, 15.85 percent. Allowing for 16 percent crossing over between *F* and *I* and the independent segregation of *I* and *C* (BATESON and PUNNETT 1908, HADLEY 1914) the expected proportions of male and female gametes in this cross are as follows:

Male gametes		Female gametes	
<i>FiC</i>	21	<i>FiC</i>	25
<i>FIc</i>	21	<i>Fic</i>	25
<i>fiC</i>	4	<i>fiC</i>	25
<i>fIc</i>	4	<i>fic</i>	25
<i>FiC</i>	4	..	..
<i>Fic</i>	4	..	..
<i>fiC</i>	21	..	..
<i>fic</i>	21	..	..
	100		100

The theoretical distribution of phenotypes derived from these gametes and that distribution to be expected if there were no linkage are given in table 2 along with the actual frequencies in each class.

TABLE 2  
*Actual and theoretical distributions of the progeny of L15.*

	FRIZZLED		NORMAL	
	WHITE*	COLOR	WHITE*	COLOR
Observed distribution	27	12	3	11
Expected (1) with 16 per cent crossing over between <i>F</i> and <i>I</i> .	28.2	11.5	4.9	8.4
Expected (2) with independent assortment.	24.8	14.9	8.3	5.0
For (1) $\chi^2 = 1.613; n = 3; P = .66$				
For (2) $\chi^2 = 11.343; n = 3; P = .01$				

\* White fowls may be *IC*, *Ic* or *ic*.

By the application of the  $\chi^2$  test for goodness of fit it is found that the value of  $P$  for the fit to the expectation with independence is so small as to preclude the possibility of independent segregation of  $F$  and  $I$  in this cross. On the other hand the  $P$  value of 0.66 indicates a good fit to the expectation with 16 percent crossing over. It is recognized that this is only an approximate measure of the amount of crossing over which actually occurred, but the  $\chi^2$  tests do eliminate the hypothesis of independence and do indicate linkage.

From all the evidence for the linkage of  $F$  and  $I$ , summarized in table 3, it is clear that these two genes are in the same chromosome and that crossing over between them amounts to about 18 percent. The crossing over in a total of 272 measurable gametes is 18.38 percent; but in determining this figure the shortcomings of a result obtained by lumping together gametes of both sexes and having the genes in both the coupling and repulsion phases, are fully recognized.

TABLE 3  
*Summary of tests for linkage of  $F$  and  $I$ .*

BIRDS TESTED	SEX	TYPE OF MATING	PHASE	GAMETES TESTED NUMBER	CROSSING OVER PERCENT
12 "K" females	♀	Backcross	Repulsion	157	19.74
3 other females	♀	Backcross	Coupling	33	18.18
A1064	♂	Backcross	Coupling	82	15.85
L15	♂	Partial backcross	Coupling	53	Approximately 16.00

#### ARRANGEMENT OF THE LINKED GENES

From the work of SUTTLE and SIPE (1932) it would appear that the genes for frizzling and crest are about 28 crossover units apart. So far as can be ascertained from their data, it is not clear that all the  $FfC_r c_r$  females used in their final crosses had the two dominant genes in the coupling

phase. If some of these were  $\frac{F C_r}{f C_r}$  and others  $\frac{F C_r}{f c_r}$  (as seems possible from

the fact that these birds were apparently two or more generations removed from the original frizzled, crested parent), the resultant distribution would not give a true measure of crossing over. However, since the percentage of crossing over in these females was almost the same as in a male which

was evidently  $\frac{F C_r}{f c_r}$ , the data for the females are evidently valid, and it

may be accepted that  $F$  and  $C_r$  are about 28 units apart.

This being the case, and the gene *I* being about 18 units from *F*, it follows that *I* and *C<sub>r</sub>* should be either closely linked (about 10 units apart) or very loosely linked (depending upon whether *I* lies between *F* and *C<sub>r</sub>*, or to the left of both). In the latter case, the crossing over to be expected between *I* and *C<sub>r</sub>* would be 46 percent, less whatever reduction would be caused by double crossing over. The only evidence so far available on this point is found in a small *F<sub>2</sub>* generation involving *I* and *C<sub>r</sub>*, reported by DUNN and JULL (1927) in which the segregation approached that to be expected if the genes were independent. The same authors mention (p. 32) a backcross of an *IiC<sub>r</sub>c<sub>r</sub>* male to Brown Leghorn females but report only the ratio of crested to non-crested among the offspring. If *I* and *C<sub>r</sub>* were closely linked, the fact would surely have been noticed in this mating, whereas crossing over of more than 40 percent would have been almost indistinguishable from independent assortment except in larger numbers than they had. It therefore seems probable that *I* and *C<sub>r</sub>* are very loosely linked and that *F* lies between them.

The data thus far available do not definitely establish the relationships between dominant white and cerebral hernia, but DUNN and LANDAUER (1930) have shown that the *I* and *h<sub>e</sub>* are very closely linked. Unfortunately the expression of *h<sub>e</sub>* is somewhat irregular in any but specially selected material so that it was impossible for these investigators to determine more exactly the linkage relations of the genes with which they worked. In one backcross in which the distribution indicates that *h<sub>e</sub>* was fully expressed, crossing over between *I* and *h<sub>e</sub>* was about 11 percent.

In consideration of the findings reviewed above and pending further data, it seems not unlikely that the four genes in this chromosome are arranged in the following order: *I*, *h<sub>e</sub>*, *F* and *C<sub>r</sub>*, and spaced somewhat as is suggested in figure 1. The gene *h<sub>e</sub>* may be on either side of *I*.

It is desirable that some gene be located between *F* and *C<sub>r</sub>* to permit working with shorter map distances in this region, and also that all the existing data be confirmed and extended. Experiments in these directions are in progress at this laboratory.



FIGURE 1.—Tentative arrangement of genes in the *IFC<sub>r</sub>* chromosome.

#### GENES NOT IN THE *IFC<sub>r</sub>* CHROMOSOME

To facilitate further progress in mapping the chromosomes of the fowl it is desirable to report all genes which have thus far been tested and found to show no linkage with those in the *IFC<sub>r</sub>* chromosome. (Students of the genetics of maize have found it preferable to label the chromosomes

of that species by some distinctive gene or genes, rather than as chromosomes I, II, III, etc. For the present, their example seems well worth following.)

### *Rose comb (R)*

In 46 progeny from two *FfRr* females backcrossed to an *ffrr* male the following distribution was found.

<i>Frizzled rose</i>	<i>Frizzled single</i>	<i>Normal rose</i>	<i>Normal single</i>
13	9	11	13

The numbers are small but the class frequencies suggest independence.

Better evidence that *R* is not in the *IFC*<sub>7</sub> group was obtained indirectly by LANDAUER's (1932) finding of no linkage between frizzling and creeper (table 6). Since creeper is very closely linked to *R* (LANDAUER 1931), the elimination of creeper should automatically eliminate *R* from this linkage group. It is therefore probable that the excess of parental combinations observed by DUNN and JULL (1927) in a backcross of rose, crested males to single, non-crested females (table 6) merely represented a chance deviation from the expected ratio. *R* and *I* were independent in their data.

### *White Shanks (W)*

Evidence that *F* and *W* are independent was obtained in five matings involving these two genes (table 4).

Although the deviations from expectation in the totals for the backcrosses are fairly large, it seems likely that they arose from chance deviations in the progenies of the two males. In these some error may have been incurred by classifying some of the birds before maturity. The dis-

TABLE 4  
*Segregation of F and W in five matings.*

TYPE OF MATING	FRIZZLED		NORMAL	
	WHITE SHANKS <i>FW</i>	YELLOW SHANKS <i>Fw</i>	WHITE SHANKS <i>fW</i>	YELLOW SHANKS <i>fw</i>
1. Backcross ♀♀ <i>FfWw</i> × ♂♂ <i>ffww</i> .	9	8	8	11
2. Backcross ♂♂ <i>FfWw</i> × ♀♀ <i>ffww</i> .	12	14	12	5
3. Backcross ♂♂ <i>FfWw</i> × ♀♀ <i>ffww</i> .	2	11	15	7
Totals	23	33	35	23
<i>Expected with independence</i>	28.5	28.5	28.5	28.5
4. <i>F</i> <sub>2</sub> observed	24	9	9	2
<i>Expected with independence</i>	24.75	8.25	8.25	2.75
5. Partial backcross, ♂♂ <i>FfWw</i> × ♀♀ <i>Ffww</i>	11	5	2	6
<i>Expected with independence</i>	9	9	3	3



tributions in matings 4 and 5 are based on adult classifications and fit the expectations fairly well. More data are desirable but so far these two genes appear to be independent.

This is confirmed by the independent segregation of dominant white and white shanks in reciprocal backcrosses and in a partial backcross (table 5).

TABLE 5  
*Segregation of I and W in three matings.*

TYPE OF MATING	WHITE*		COLORED	
	WHITE	YELLOW	WHITE	YELLOW
	SHANKS W	SHANKS w	SHANKS W	SHANKS w
1. Backcross $\sigma^7 IiWw \times \text{♀ } iiww$	17	11	7	8
2. Backcross $\text{♀ } iiWw \times \sigma^7 Iiww$	10	3	6	4
Totals	27	14	13	12
<i>Expected with independence</i>	16.5	16.5	16.5	16.5
3. Partial backcross $\sigma^7 IiWwCc \times \text{♀ } iiWwCc$	17	6	14	5
<i>Expected with independence</i>	19.7	6.6	11.8	3.9

\* In mating 3 white fowls may be *IC*, *Ic* or *ic*.

If the excess in the *IW* class of the backcross progeny were accompanied by a corresponding excess in the *iw* group, linkage would be indicated, but the deficiency in the latter class (table 5) suggests that the excess of *IW* birds can be ascribed to chance or, more likely, to errors in classification. The distribution in mating 3 is based upon descriptions of adults and fits the expectation remarkably closely.

From these two sets of data it is evident that *W* is either not in the *IFC*, chromosome, or, if it be present, too far removed from both *I* and *F* to show linkage with either of them.

### *Other Genes*

Evidence that fourteen characters are independent of the *IFC*, group is summarized in table 6.

As is to be expected, characters showing no linkage with one member of the group have also shown independence with other members of the *IFC*, chromosome. It may be noted that the possibility that polydactyly belongs to this linkage group, as was suggested by DUNN and JULL (1927), is not excluded by the data thus far available. The combined backcross ratio of parental to new combinations when polydactyly was tested with dominant white was 213:186 and the corresponding ratio in trials with crest was 281:243. The question probably will not be settled till some gene is located to the left of *I* in the chromosome, so that the possibility

TABLE 6

*Characters apparently independent of the IFC, group.\**

LINKED GENE	TESTED WITH	INVESTIGATOR	EVIDENCE FOR INDEPENDENCE
<i>I</i>	Recessive white (or black)	BATESON and PUNNETT (1908)	Independence in 77 back- cross and in 67 F <sub>2</sub>
<i>I</i>	Recessive white (or black)	HADLEY (1914)	Independence in 167 F <sub>2</sub>
<i>I</i>	Rumplessness	DUNN (1926)	No linkage in 231 progeny
<i>I</i>	Rumplessness	WARREN (1932)	Backcross 84:104
<i>I</i>	Rose comb	DUNN and JULL (1927)	Independence in 170 F <sub>2</sub> Backcross 31:28
<i>I</i>	Rose comb	WARREN (1932)	Backcross 88:91
<i>I</i>	Pea comb	WARREN (1932)	Backcross 285:262
<i>I</i>	Polydactyly	SEREBROVSKY and PETROV (1930)	Backcross 52:37
<i>I</i>	Polydactyly	WARREN (1932)	Backcross 161:149
<i>I</i>	Creeper	SEREBROVSKY and PETROV (1930)	Backcross 96:80
<i>I</i>	Silky	DUNN and JULL (1927)	Independence in 93 F <sub>2</sub>
<i>I</i>	White shanks	HUTT (this paper)	See table 5
<i>I</i>	White shanks	WARREN (1932)	Backcross 122:107
<i>I</i>	Leg feathering	WARREN (1932)	Backcross 157:137
<i>I</i>	Naked neck	WARREN (1932)	Backcross 304:308
<i>F</i>	Black	SEREBROVSKY and PETROV (1930)	Backcross 63:50
<i>F</i>	Blue	SEREBROVSKY and PETROV (1930)	Backcross 79:88
<i>F</i>	Split comb	SEREBROVSKY and PETROV (1930)	Backcross 33:40
<i>F</i>	Split comb	LANDAUER (1932)	Backcross 84:91
<i>F</i>	Rose comb	SEREBROVSKY and PETROV (1930)	Backcross 24:24
<i>F</i>	Rose comb	HUTT (this paper)	See page 89
<i>F</i>	White shanks	HUTT (this paper)	See table 4
<i>F</i>	Creeper	LANDAUER (1932)	Backcross 97:96
<i>F</i>	Naked neck	LANDAUER (1932)	Backcross 116:117
<i>F</i>	Mesodermal pigment	SEREBROVSKY and PETROV (1930)	Backcross 39:35
<i>C<sub>r</sub></i>	Silky	DUNN and JULL (1927)	Fair fit to independence in 62 F <sub>2</sub>
<i>C<sub>r</sub></i>	Split comb	SEREBROVSKY and PETROV (1930)	Backcross 225:203
<i>C<sub>r</sub></i>	Pea comb	SEREBROVSKY and PETROV (1930)	Backcross 177:173
<i>C<sub>r</sub></i>	Rose comb	DUNN and JULL (1927)	Backcross 110:75
<i>C<sub>r</sub></i>	Rose comb	WARREN (1932)	Backcross 80:92
<i>C<sub>r</sub></i>	Rose comb	SEREBROVSKY and PETROV (1930)	Backcross 336:282
<i>C<sub>r</sub></i>	Blue	SEREBROVSKY and PETROV (1930)	Backcross 120:119
<i>C<sub>r</sub></i>	Black	SEREBROVSKY and PETROV (1930)	Backcross 222:215
<i>C<sub>r</sub></i>	White shanks	SEREBROVSKY and PETROV (1930)	Backcross 87:73
<i>C<sub>r</sub></i>	White shanks	WARREN (1932)	Backcross 107:115
<i>C<sub>r</sub></i>	Muff and beard	SEREBROVSKY and PETROV (1930)	Backcross 388:328
<i>C<sub>r</sub></i>	Rumplessness	WARREN (1932)	Backcross 112:122
<i>C<sub>r</sub></i>	Naked neck	WARREN (1932)	Backcross 125:107
<i>C<sub>r</sub></i>	Leg feathering	WARREN (1932)	Backcross 115:119
<i>C<sub>r</sub></i>	Polydactyly	SEREBROVSKY and PETROV (1930)	Backcross 160:130
<i>C<sub>r</sub></i>	Polydactyly	WARREN (1932)	Backcross 121:113

\* The ratio given in backcross data is that of parental combinations to new combinations.

of polydactyly being near the extreme left end may be examined, unless the character be first found linked to some other which is definitely known to be independent of this group.

SEREBROVSKY and PETROV (1930) have reported a considerable number of linkage trials involving *I*, *F*, or *C<sub>r</sub>*, but some of them had numbers too few to indicate either linkage or independence and for that reason not all of their data are included in table 6. Additional evidence that genes for leg feathering and mesodermal pigment are not in the *IFC<sub>r</sub>* group was provided by DUNN and JULL (1927), chiefly in *F<sub>2</sub>* data. These workers also found several characters independent of cerebral hernia, but since *h<sub>e</sub>* was somewhat irregular in its appearance in their stocks, and since DUNN and LANDAUER (1930) found it closely linked with *I*, it seems unnecessary to present their data. Genes independent of *I* are bound to be independent of *h<sub>e</sub>*.

The association between crest and the trifold or multiple-point condition of the rose comb is well known. In 545 rose-combed birds raised by JULL (1930) from silky crosses, there were only 12 exceptions to the rule that crested birds had multiple point combs and non-crested fowls had single-spike combs. JULL concluded that the twelve had been incorrectly classified and that there is not close linkage between crest and some factor for multiple points but rather that the rose comb is modified by the gene for crest.

#### THE INFLUENCE OF SEX ON CROSSING OVER

To the best of the writer's knowledge, the first definite evidence that crossing over occurs in the female fowl was found by DUNN and LANDAUER

(1930) in a backcross of  $\frac{I H_e}{i h_e}$  females to an *ii h<sub>e</sub>h<sub>e</sub>* male. This was to be

expected in view of the previous finding by CHRISTIE and WRIEDT (1923) that crossing over occurs in the autosomes of the pigeon, which suggested that in birds female digamety is not a bar to crossing over as it is in the silkworm. The numbers observed in either of these cases were hardly adequate for the measurement of the effect of sex on crossing over in birds.

The data thus far available are not sufficient to permit conclusions about sex differences in crossing over in the fowl but it is of interest to note that males thus far tested have displayed slightly less crossing over than females. The figures are:

	Genes	Crossing over percentage	
		in ♂♂	in ♀♀
LANDAUER (1931)	<i>C<sub>r</sub></i> * and <i>R</i>	0.19 (2136)	0.68 (2347)
SUTTLE and SIPE (1932)	<i>F</i> and <i>C<sub>r</sub></i>	27.16 (81)	29.87 (77)
HUTT (this paper)	<i>I</i> and <i>F</i>	15.85 (82)	19.47 (190)

\* Creeper.

The numbers in parentheses give the number of gametes tested in each case. Pending further data it would appear that the effect of sex on crossing over is somewhat the same in the fowl as in the rat and the mouse. In these mammals crossing over is somewhat lower in the male than in the female.

If further data on crossing over in the two sexes of the fowl should confirm those given above, it would then be apparent that sex differences in crossing over do not depend upon heterogeneity of the sex chromosomes but upon other conditions associated with sex. In this connection it is of interest to point out that in the locust, *Paratettix*, and the crustacean, *Gammarus*, crossing over is also lower in males than in females, and that it is not found at all in the male of *Drosophila*. The absence of crossing over in the female silkworm is apparently at variance with the situation in other species thus far investigated.

#### SUMMARY

Crossing over between the genes for frizzling and for dominant white was found to be about 18 percent.

This finding along with those of other workers permits the tentative assignment to one linkage group of the genes for dominant white, cerebral hernia, frizzling and crest, and gives some idea of the arrangement of these genes in the *IFC'* chromosome.

It was found that the genes for rose comb and for white shanks are not in this linkage group and the available evidence for the independence of other genes is presented.

Evidence that in the fowl crossing over is less in males than in females is considered and it is suggested that sex differences in crossing over may depend, not on heterogeneity of the sex chromosomes, but upon other conditions associated with sex.

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# EMBRYO DEVELOPMENT IN NICOTIANA SPECIES HYBRIDS

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## INTRODUCTION

In a study of the compatibilities of certain *Nicotiana* species, several crosses were reported which produce seeds unable to germinate. (McCray 1932). Many others are recorded in the literature on this genus. Some of these seeds are parthenocarpic; others have embryos not able to develop. In still other cases the hybrid germinates but is unable to carry on further. All degrees of vigor have been observed from these conditions up to heterosis. Several interesting questions might be raised in connection with the development of the hybrid embryos displaying such different degrees of viability and vigor. Does the hybrid begin its development as soon after pollination as does the new individual of pure species? That is to say, does fertilization take place as readily in one case as in the other? Do the cell divisions in the embryo and its consequent growth proceed as rapidly in the hybrid as in the pure species? If so, when does growth in the non-viable embryos stop? If not, what sort of growth rate does the hybrid have as compared with its pure parent? These are the questions which the present study attempts to answer.

Strangely, considering all the work which biologists, especially botanists, have done on hybridization, the scientific literature sheds very little light on these problems. It is nearly an unworked field. So far as I have been able to find only two studies have any bearing on the problem in hand, those of KOSTOFF (1930) and BRIEGER (1928), both on *Nicotiana*.

To be sure KRUMHOLZ (1930) compared the size of embryos of interspecific *Oenothera* hybrids with that of embryos of the pure species, but he measured only the fully matured embryos, and says nothing about their growth. Incidentally it might be noted here that he found, in reciprocal crosses, the embryo size to be practically determined by the female used in the cross. If the female was of a large seeded species, the hybrid embryo was large and *vice versa*.

KOSTOFF describes several experiments on the formation and development of hybrid embryos. He points out the fact that in some crosses the embryo develops for a few (up to 15) days, then stops, followed by the death of the ovule; he mentions other cases in which the young hybrid persists for a longer period, although not able to mature to the point where germination is possible. He also observed that parthenocarpic seeds and

those with small embryos are always smaller than seeds resulting from selfing. The sizes of several kinds of interspecific hybrid embryos of *Nicotiana* are recorded.

On the point of rate of growth this author states only that, with respect to certain crosses, "the embryos begin to develop quite normally, but when compared with the embryos obtained after selfing, a retardation of the growth of the hybrid embryo took place a few days after fertilization. The time of the retardation of the embryonic growth depends, of course, on the cross combination. This retardation gradually increases with the embryonic period of growth."

These observations give a very inadequate picture of the comparative growth rates. On the other hand, however, this investigator made a partial study of causes of failure of the hybrid to grow normally, and of some other effects of the hybrid condition. More specifically, he discusses the formation of extra amounts of starch outside the nucellus, and some other morphological abnormalities in ovules carrying hybrid embryos.

In view of the considerable differences in different *Nicotiana* hybrids it was thought advisable, in the present study, to choose crosses representing several different degrees of vigor in the hybrid. Accordingly four different groups of cross combinations were recognized and two crosses selected to test the embryo development in each group. Group I includes hybrids which fail in their embryonic development very soon after fertilization; group II comprises others which also fail to complete embryonic growth but develop somewhat farther than those in group I. Group III hybrids grow to their full embryonic size, give a partial germination, but are weak and unable to mature. Those other more successful combinations which do mature constitute group IV.

The crosses to represent the first two groups were chosen largely on the strength of KOSTOFF's data. They are, for group I, *N. rustica* × *N. glauca* and *N. rustica* × *N. longiflora*; for group II, *N. rustica* × *N. Rusbyi* and *N. rustica* × *N. Palmeri*. In each of these crosses the *N. rustica* var. *brasilia* was used.

*N. nudicaulis* × *N. Tabacum* and *N. suaveolens* × *N. Tabacum* are of group III. CHRISTOFF (1928) reports both of these crosses as being weak, and EAST (1928) and KOSTOFF concur in the observation on the latter. I myself tested them also. Of 100 seeds of *N. nudicaulis* × *N. Tabacum* 7 germinated but died a few days later. Of a later planting of 68 seeds 10 germinated and again died in the cotyledon stage. *N. suaveolens* × *N. Tabacum* gave a strong germination, but 10 plants which were transferred to small pots all died at the same young seedling stage. KOSTOFF tested many seedlings with identical results. In both of these crosses I used the *N. Tabacum* var. *purpurea*.

EAST and KOSTOFF have each grown to maturity plants of *N. Tabacum*  $\times$  *N. glauca*. *N. paniculata*  $\times$  *N. glauca* is reported by EAST, and by GOOD-SPEED and CLAUSEN, as having matured hybrid plants (EAST 1928). These two hybrids, which I also found vigorous in my own tests, represent group IV. In the former of these crosses I used the *N. Tabacum* var. *sanguinea*.

#### MATERIALS AND METHODS

Because of a possible bearing on the results obtained it is first to be noted that the pollinations for the series of observations to be reported here were made over a period of several months, from October to May. It might have been preferable to make them within a shorter period so that all the embryos would have been subjected to more nearly the same environmental conditions, especially temperature and length of days, but under the circumstances this was impossible. This point will be discussed later.

The plants used were either obtained from Bussey Institution, HARVARD UNIVERSITY, where the work was done, or grown from seed I had saved in the course of earlier experiments on the same species already referred to. All the plants were kept in a screened greenhouse, the pollinations were made by hand, and the usual precautions were taken to prevent accidental crossing.

The capsules were fixed in Allen's modification of Bouin's fluid, B-15, and stained in Haidenhain's haematoxylin.

The developing capsules were fixed at intervals of two days, so the embryo lengths were recorded at 7, 9, 11, 13, 15, 17, 19, and 21 days after pollination. It was thought that by 21 days after pollination the embryos would have practically attained their full size, as the seeds often ripen at about 25 days. This apparently is not quite true as they continued, in some instances, to increase in length rather rapidly up to 21 days, indicating that there would have been still further growth had the capsules not been removed. However the embryos were sufficiently matured by this time to show clearly the difference in development between the hybrid and its maternal parent, which is the point in which we are especially interested.

Because of the desirability of measuring several embryos of each age, the whole capsules were fixed and embedded in paraffin, so that a number of ovules were sectioned and mounted on each slide. Considerable difficulty was encountered in the sectioning from two causes, (1) incompleteness of infiltration of paraffin into the ovules, especially when nearing maturity, and (2) the ease with which the ovules were broken loose from the placenta, and the embryos removed from the ovules. Fortunately the



embryos are quite strong and usually remain intact even when they are pulled out of place by the knife.

To obviate this latter difficulty I experimented with cedar oil as a clearing agent to prevent some of the hardening effects of alcohol and xylol in the process of dehydration and clearing. I also tried ZIRKLE'S (1930) butyl alcohol procedure. This seemed to give somewhat better results and was followed in most of this work. KRUMBHOLZ (1930) soaked his *Oenothera* seeds in water until they were soft and then removed the embryos before fixing. But he was dealing with only the matured seeds which simplified the problem in this regard. A half and half mixture of hard and soft paraffin seemed to facilitate the sectioning somewhat as compared with the soft paraffin.

The embryos were measured with an eyepiece micrometer. This procedure is simple enough, except when the embryos are quite sharply curved, as are the older ones in some instances. In these cases the curved axes of the embryos were divided into a number of short arcs, each one approximating a straight line, and the ocular gradually rotated so the scale was brought into contact with these arcs successively throughout the length of the embryo. Sometimes in the older embryos there was a difference in the measurement depending on which side of the embryo was measured. That is to say the cotyledons were not of the same length. In these cases the average of the two measurements was recorded. Practically all the embryos were measured in such position that the two cotyledons lay side by side at about the same level. In the latter part of the work a few measurements were taken from the "side view" of the embryo, one cotyledon lying beneath the other.

The number of measurements at each age varied from 1 to 20, the average number being  $9 \frac{2}{3}$ . In only 8 cases, however, were less than 5 embryos measured, and in half the cases 10 or more measurements were averaged together. Altogether 761 embryos are included in the data.

#### OBSERVATIONS AND RESULTS

No fusions of egg and sperm were seen in the course of these observations, but in one preparation of *N. rustica*  $\times$  *N. glauca* at 7 days and in another of *N. Tabacum* at 7 days, I found fertilized egg cells which had undergone only one division, as shown in figure 1a. In several instances the first measurements recorded were made on single cell embryos. It therefore seems quite clear that fertilization usually took place on the seventh day,—or on the ninth day in two instances. An exception is seen in *N. rustica*. The 7 day embryos in this case are spherical, much larger than the others, and have about 32 cells.

Although not particularly pertinent to the results of the study it is of

some interest to note the changes in shape of the embryo as it develops. There are minor differences in embryo shape in the different species used, but in general the process of development is about as follows. Following

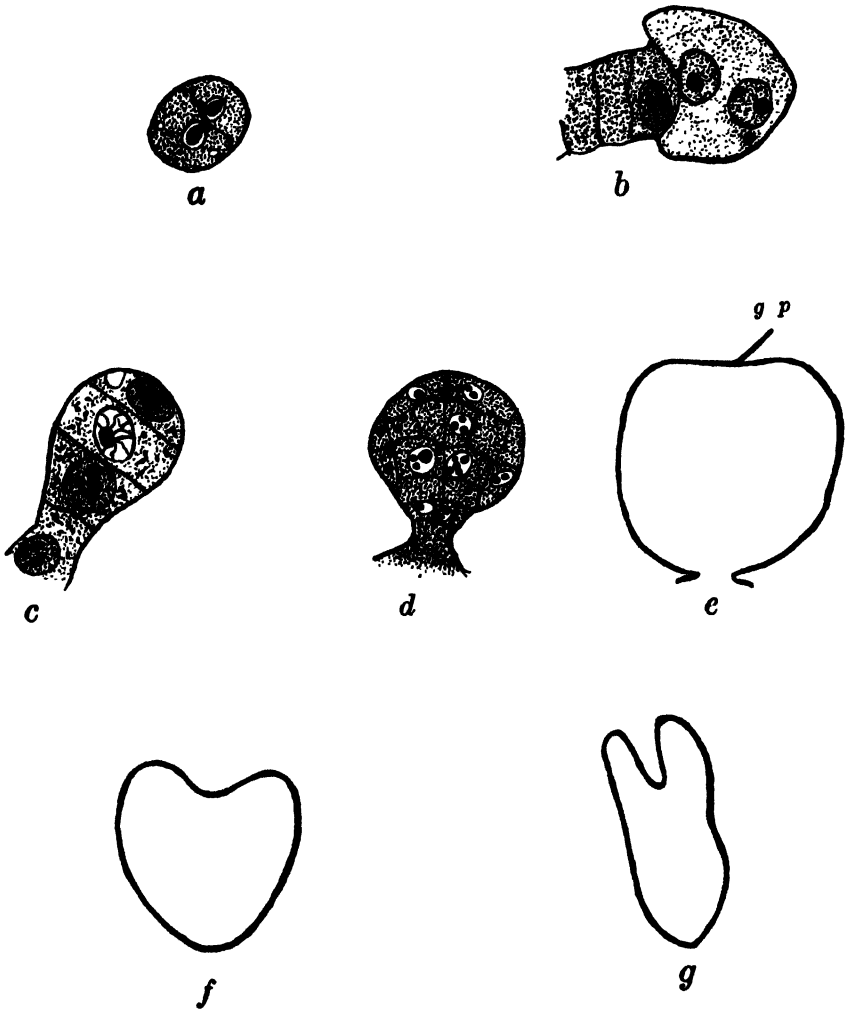


FIGURE 1.—Embryo development in *Nicotiana* species, e, f and g diagrammatic, showing shape only. a. Fertilized egg cell in first division, *N. rustica*  $\times$  *N. glauca*, 7 days after pollination ( $\times 360$ ). b. Embryo at end of suspensor, containing two nuclei, but cell not yet divided, *N. paniculata*, 7 days ( $\times 810$ ). c. *N. suaveolens* embryo in 3 cell stage, 7 days ( $\times 360$ ). d. *N. rustica* var. *brasilia* in about 32 cell stage, 7 days ( $\times 432$ ). e. *N. rustica* var. *brasilia*, 11 days. g.p., growing point ( $\times 432$ ). f. *N. paniculata*, 13 days ( $\times 330$ ). g. *N. paniculata*, 15 days ( $\times 110$ ).

fertilization the new zygote undergoes several divisions, resulting in a linear series of cells, as is common in Angiosperms. The distal end of the chain is the embryo proper, the others constituting the pro-embryo or suspensor, which attaches the embryo to the micropylar end of the ovule

(figure 1b). The semicircular embryo cell then enters upon its long series of cell divisions which eventually may bring it to maturity. The first divisions are transverse so the linear series of cells is extended (figure 1c). As cell divisions ensue in other planes, the embryo gradually assumes a spherical shape as in figure 1d. Within a few days the distal end of the embryo has widened out so that the longitudinal section presents a wide rounded wedge (figure 1e). By this time the cells have undergone some differentiation, and the growing point is located, as shown in the figure, in the middle portion of the distal end. As the cotyledons begin to appear the two sides of the wedge lengthen until the embryo has a distinctly forked appearance which it retains to maturity (figures 1f and g).

All the average embryo lengths are shown in table 1, in millimeters, and the same results are shown graphically in figures 2 to 6.

TABLE 1  
*Growth in length of embryos of Nicotiana species and their hybrids (measured in mm).*

DAYS FROM POLLINATION	<i>rustica</i> VAR. <i>brasilia</i>	SPECIES OR HYBRID				<i>nudicaulis</i>	<i>nudicaulis</i> × <i>Tabacum</i> VAR. <i>purpurea</i> III
		<i>rustica</i> × <i>glauca</i> I	<i>rustica</i> × <i>longiflora</i> I	<i>rustica</i> × <i>Rusbyi</i> II	<i>rustica</i> × <i>Palmeri</i> II		
7	0.0481	0.0209	0.0136	..	0.0416	0.0234	0.0234
9	0.1569	..	..	0.0435	0.0447	0.0446	0.0207
11	0.1214	..		0.0370	0.0444	0.0360	0.0333
13	0.2303	..		.	0.0574	0.0592	0.0567
15	0.4435	..				0.0576	0.1070
17	0.6375		.	.		0.1010	0.1551
19	0.8006	.	.			0.1126	0.0843
21	0.9020	..	.		.	0.4572	0.3152

DAYS FROM POLLINATION	<i>suaveolens</i>	SPECIES OR HYBRID			<i>paniculata</i>	<i>paniculata</i> × <i>glauca</i> IV
		<i>suaveolens</i> × <i>Tabacum</i> VAR. <i>purpurea</i> III	<i>Tabacum</i> VAR. <i>sanguinea</i>	<i>Tabacum</i> VAR. <i>sanguinea</i> × <i>glauca</i> IV		
7	0.0296	0.0281	0.0182	..	0.0210	0.0111
9	0.0390	0.0470	0.0291	0.0185	0.0244	0.0204
11	0.0807	0.0539	0.0412	0.0345	0.0838	0.0616
13	0.0574	0.0662	0.0668	0.0395	0.0903	0.0631
15	0.1194	0.1117	0.2216	0.0812	0.2777	0.0722
17	0.7655	0.2888	0.4880	0.2749	0.3915	0.1389
19	0.6769	0.6383	0.4945	0.3246	0.3930	0.1820
21	0.5765	0.7625	0.5183	0.4087	0.4413	0.2563

The hybrids of group I, *N. rustica* × *N. glauca* and *N. rustica* × *N. longiflora*, made almost no growth at all. Measurements were secured only at 7 days. Then the embryos apparently died and the ovules degenerated. The appearance of such a dead ovule is indicated in figure 7.

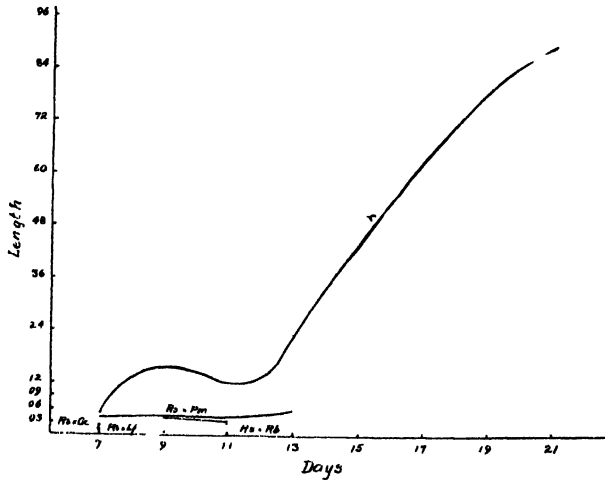


FIGURE 2.—Growth curves of embryos. *Rs*, *N. rustica* var. *brasilia*; *Rs*×*Gc*, *N. rustica*×*N. glauca*; *Rs*×*Lf*, *N. rustica*×*N. longiflora*; *Rs*×*Rb*, *N. rustica*×*N. Rusbyi*; *Rs*×*Pm*, *N. rustica*×*N. Palmeri*.

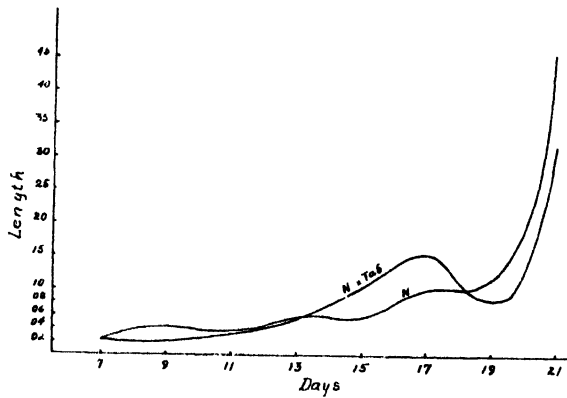


FIGURE 3.—Growth curves of embryos. *N*, *N. nudicaulis*; *N*×*Tab*, *N. nudicaulis*×*N. Tabacum*.

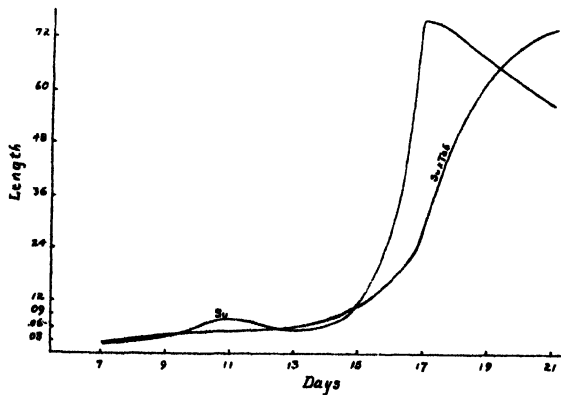


FIGURE 4.—Growth curves of embryos. *Su*, *N. suaveolens*; *Su*×*Tab*, *N. suaveolens*×*N. Tabacum*.

The hybrids of group II persisted somewhat longer, but they also failed to make any appreciable growth. *N. rustica* × *N. Rusbyi* was observed only at 9 and 11 days, and *N. rustica* × *N. Palmeri* died after the thirteenth day (figure 8).

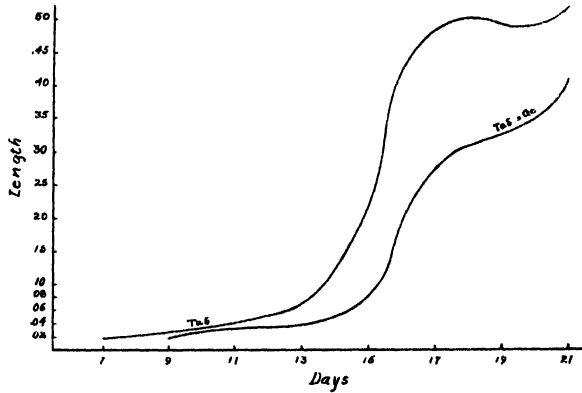


FIGURE 5.—Growth curves of embryos. Tab, *N. Tabacum* var. *sanguinea*; Tab × Gc, *N. Tabacum* var. *sanguinea* × *N. glauca*.

While our chief interest might be in the unsuccessful hybrids of the preceding groups, there is less to say of them than of the others. They seemed doomed from the start. Whatever the nature of the incompatibility exhibited they are not able to grow at all.

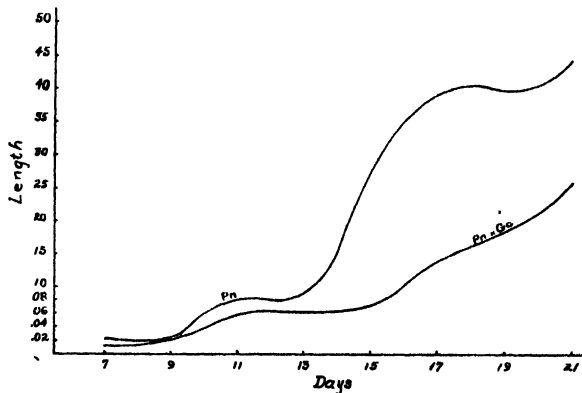


FIGURE 6.—Growth curves of embryos. Pn, *N. paniculata*; Pn × Gc, *N. paniculata* × *N. glauca*.

In all the other crosses the full number of eight observations was made except in *N. Tabacum* × *N. glauca* where also the embryo made its first appearance at 9 days. The progress of the hybrids of groups III and IV can, naturally, best be studied from the growth curves. A general similarity between the curves will at once be apparent, especially if we omit for the present those representing *N. suaveolens* (figure 4), *N. nudicaulis*

and *N. nudicaulis* × *N. Tabacum* (figure 3). These three curves will be discussed presently.

#### DISCUSSION

PEARL (1925) has brought together data from many sources and shown that many growth curves, based on such different forms of life as rats, pumpkins, and tadpoles, and even curves representing growth in populations of yeast, flies and human beings, assume much the same form. He concludes that wherever growth is based on multiplication of cells a similar logistic, S-shaped curve, first employed by VERHULST (1838) in describing population growth, and since used by ROBERTSON (1923) and others, could be used to represent the growth of the organism in question.

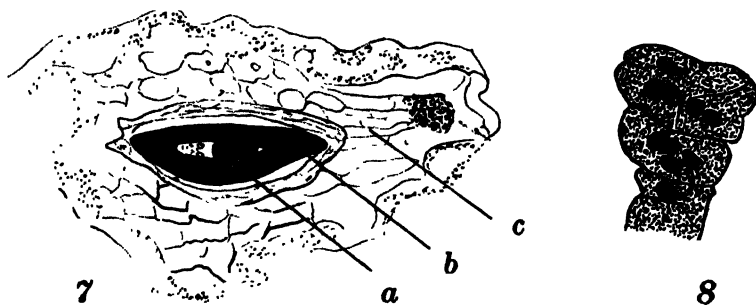


FIGURE 7.—Dead ovule, *N. rustica* var. *brasilia* × *N. glauca*, 11 days after pollination (×432).  
a. embryo; b. nucellus; c. endosperm.

FIGURE 8.—Embryo of *N. rustica* var. *brasilia* × *N. Rusbyi*, 11 days, in state of disorganization. Note shrunken appearance and larger number of nuclei than of cells (×432).

My six curves which are most alike also approximate this logistic, S-shaped curve. Especially is this true of the one representing *N. suaveolens* × *N. Tabacum* (figure 4). Apparently the same law, as might be expected, governs growth in the embryonic state as well as in later life.

PEARL also brings out the fact that the complete growth curve of an individual might cover successive cycles of growth, each of which could be represented by such a logistic curve. It seems from the data here presented that embryo development constitutes the first of such cycles, which would be followed, as illustrated by PEARL's pumpkin curve for example, by other cycles of growth after germination. The curve for *N. paniculata* (figure 6), indeed, suggests the occurrence of two cycles during the embryonic stage alone. But since this curve is an exception in that regard it is assumed that the hump in this curve at 11 days which gives it that appearance is due to the same kind of variations as those which produced a hump at 9 days in *N. rustica* (figure 2). These variations are not considered significant.

The main point for our present study is the comparison between the

hybrids in the different categories, and between each hybrid and its maternal parent species. Again a similarity is noted between the different pairs of curves—leaving out of account all the curves in figure 2 for obvious reasons. The curve for each hybrid approximates that for the mother species, but falls somewhat below the latter. The retardation in the growth rate of the hybrid becomes more noticeable at the time when the increase in growth rate becomes suddenly much greater, in other words, when the curve turns sharply upward. This is at 13 days after pollination in *N. Tabacum* and *N. paniculata* and their hybrids, at 15 days in *N. suaveolens* and *N. suaveolens*  $\times$  *N. Tabacum*, and probably still later in *N. nudicaulis* and its hybrid. This retardation becomes greater for a time, causing the curves to draw farther apart. Later they approach each other again, indicating that the hybrid embryo for a short time near the end of its development grows faster than the pure species embryo. There is always, however, in the crosses studied, a considerable difference in maximum size between the embryos of pure species and those of the corresponding hybrids.

There seems to be no difference between the growth of hybrid embryos in groups III and IV. There is nothing in the growth of group III hybrid embryos upon which to predict ultimate failure.

*N. nudicaulis* and its hybrid offspring gave less typical curves, due, in the first place, to the fact that the most rapid growth came at a later time. At 21 days when the observations were discontinued both kinds of embryos seemed to be still growing rapidly. Very likely if measurements had been made up to 25 days very much the same type of curve as the others would have resulted.

Besides, in the curve for this hybrid, *N. nudicaulis*  $\times$  *N. Tabacum*, as well as in the curve for *N. suaveolens*, there are notable irregularities. Each one crosses and recrosses its mate. The *N. suaveolens* embryos, furthermore, according to the data, reached their maximum length at 17 days and then steadily grew smaller—an impossible result, of course. Two possible explanations might be offered for these anomalies. Since the period of making observations extended over fall, winter, and spring, it might be that the embryos which grew during early fall and late spring developed faster than those which grew during the shorter, colder days of winter. Or it might be that sometimes fertilization was effected more quickly. Since age is counted from pollination and not from fertilization this, it might be thought, would give some embryos an uneven start. Possibly the age of the flower when pollinated could be the cause of uneven time of fertilization.

There is some evidence that the former of these factors has some effect. One of the most extreme cases is in *N. nudicaulis*. The average of 14 em-

bryo lengths at 19 days is recorded as 0.1126 mm. The pollinations of these capsules were made on December 31. Seven embryos developing in capsules pollinated on March 28 attained at the same age an average length of 0.4380 mm. In the same species the lengths recorded at 9 days are slightly greater than those for 11 days, very possibly due to the fact that the former group of embryos developed in April, while the latter were produced in December.

On the other hand there are a number of instances where no such effect of the season can be detected. For example, the *N. paniculata* curve is scarcely as regular as those for *N. Tabacum* or *N. suaveolens*  $\times$  *N. Tabacum*; yet the pollinations for the *N. paniculata* were all made within a nine day period, about May 1, while those for *N. Tabacum* extended from December 12 to May 14, and those for *N. suaveolens*  $\times$  *N. Tabacum* from October 31 to March 19. Altogether it seems unlikely that the season in which the embryos are produced is a very important factor in determining the size or rate of growth.

Neither is it likely that flower age at the time of pollination has contributed in any important way to the irregularities noted. In the first place, in practically every case the first signs of an embryo were found at 7 days. If some flowers were older when pollinated they were evidently not able on that account to effect fertilization any sooner, and thus to give their embryos a handicap in the race. Further, three *N. rustica* flowers on the same plant but in different stages of development were selfed on the same day to test this point. Unfortunately the capsule from the oldest flower was lost. Five embryos in the capsule from the youngest flower averaged, at 13 days, 0.6482 mm, while three in the capsule from the flower at the middle stage, as ordinarily pollinated, averaged 0.8614 mm, noticeably larger to be sure. Still the difference is probably not significant as even greater variations occurred at times in a single capsule. For example, as an extreme case, the embryo lengths in one capsule ranged from 51 to 122.5 micrometer divisions.

We can only conclude that the irregularities seen in the data are caused by variations due to causes not discovered. There must be other factors at least equally potent in producing these variations with the two already postulated.

Lest the reader be misled it should be noted that by no means all the observations were as erratic as those just cited. If they had been still greater irregularities must have appeared in the curves. A typical set of measurements, in micrometer divisions, for comparison of variability shown, is as follows: 59, 44, 46, 54.5, 58.5, 45.5, 45.5, 58.25, 48.5, 53, 54, 60, 56. These are the measurements of 13 different *N. nudicaulis*  $\times$  *N. Tabacum* embryos at 21 days.



The causes of the failure of the crosses of groups I and II to grow at all, and of the failure of group III hybrids to continue growth much beyond germination, do not fall within the scope of the present investigation. The only explanations offered are those postulated by KOSTOFF, who suggests immunological relations between the maternal parent and the hybrid embryo which it supports, as one explanation of the incompatibility of species. He also observed a large accumulation of starch outside the nucellus in the ovules containing hybrids between incompatible species, which, he thinks, makes it so difficult for the embryo to get its nourishment that growth can not be carried on.

Certainly there is nothing in the results here presented to support the idea of immunological relations. On the contrary my observations are rather against this view. In the first place several hybrids made no increase in size at all. They merely existed for from 1 to 4 days, making in some cases a number of cell divisions. There seems to be something inherently wrong with the genotype here which does not permit the cells to grow. If one may draw an analogy from serological reactions in animals it would seem that if the embryos fail because of immunological relations, they should make a normal start, the injurious effects of the reaction with the maternal tissues coming only at a later time. Further, this sort of reaction can hardly explain the experience of embryos of the hybrids of group III which apparently complete their development normally, but fail in the seedling stage.

BRIEGER'S (1928) work, like mine, deals with the time of the failure of the hybrid. This writer, however, determines the time in terms of the differentiation of plant tissues, rather than physical size and length of life. His conclusions are summarized as follows: three critical phases occur where the organization of the individual may break down as the result of the incompatibility of the two genotypes: (1) formation of the vegetative growing point and differentiation of vegetative parts in the embryo; (2) differentiation of various floral parts; (3) differentiation of the archesporium or of the haploids from the archesporium.

Brief comment only need be made with reference to the second and third conclusions, which have less to do with the subject in hand, namely embryo development; then we may examine somewhat more fully the findings summarized under number 1. Finally I shall attempt a somewhat more complete analysis of the time of failure of hybrids in *Nicotiana* than these statements give.

The hybrids which in BRIEGER'S observations failed to develop the floral parts were interspecific *Nicotiana* hybrids of the  $F_2$  generation and backcrosses. He also quotes HERIBERT-NILSSON (1918) as having observed in  $F_1$  *Salix caprea*  $\times$  *S. viminalis* the substitution of vegetative leaves for

inflorescences. But in *Nicotiana* those  $F_1$  hybrids at least which have been able to complete their vegetative growth also produce flowers of the usual type.

BRIEGER's third conclusion refers to the inability of many hybrids to complete sporogenesis in such a way as to produce functional gametes. This is a phenomenon familiar to botanists generally.

The first part of his summary is correct, I am sure, as regards the fate of certain hybrids, but certainly not all. He pictures a small embryo of the backcross (*N. Tabacum*  $\times$  *N. Rusbyi*)  $\times$  *N. Tabacum* var. *Cuba* which is evidently stopped in its growth at a stage comparable to that at which my  $F_1$  hybrids of group II ceased their growth (figure 8). This seems to be, indeed, just before or at the time the growing point, located just between the two cotyledons, appears to be differentiated. But my hybrids of group I, unlike these, were able to undergo very few cell divisions and died surely before any differentiation of cells was initiated. KOSTOFF (1930) made similar observations on his cross *N. paniculata*  $\times$  *Petunia violacea*, in which case the embryos stopped in the 4-6 cell stage. In still other cases, as in my group III, the embryo completes a seemingly normal development and is still wholly unable to get beyond the seedling stage.

KOSTOFF's analysis of the situation is more complete than BRIEGER's. He puts the results of *Nicotiana* species crosses into five classes.

1. Crosses in which the pollen tubes do not reach the ovary.
2. Crosses in which the pollen tubes reach the ovary, penetrate the micropyle, and induce parthenocarpy.
3. Crosses in which fertilization occurs, but in which the embryos die in a very early stage.
4. Crosses in which the seeds germinate, but the plants die in the seedling stage.
5. Crosses from which mature hybrids are obtained.

As he points out the lines of demarcation are not sharp and there is some overlapping between these categories. It is a helpful device, nevertheless, and from the evidence already presented and the experience of other investigators, three or four other classes could be recognized. KOSTOFF's group 2 fails to distinguish between my hybrids of groups I and II,—hybrids which make only one or two divisions in the embryo, and those which live and produce a considerable number of cells over a period of about one fourth of the usual period of embryonic development. KOSTOFF himself observed some embryos of this type which developed farther than mine, in some cases to 100 cells, in *N. Tabacum*  $\times$  *N. Langsdorffii*. There are other cases in which the embryo apparently completes its growth but is unable in most cases to germinate. His classification also omits mention

of the dwarf plants. Doctor E. M. EAST informs me that these form a quite conspicuous group among his *Fragaria* hybrids, and similar dwarfs have appeared in other genera. These dwarfs, which stop at several rather definite stages of vegetative growth, are still able to bloom. And, lastly, the fact that some vigorous hybrids are fertile while others are sterile justifies making two groups out of KOSTOFF's number 5.

A more complete classification would be:

1. Crosses which produce no stimulation of the ovary with the result that the flower falls. Many such cases were noted in my experience in testing compatibilities of 23 species, as well as in the work of others. KOSTOFF found in his study of crosses of this kind that the pollen tube never entered the ovary, and that it must enter to effect the results given in any of the following groups.

2. Crosses in which the ovary is stimulated but without production of a hybrid embryo.

- a. Stimulation stops with inducing some cell divisions and growth in the nucellus (KOSTOFF).

- b. Parthenocarpic seeds are produced.

- c. Parthenogenetic (maternal) seeds are produced, either haploid or diploid.

3. Crosses in which true hybrids are produced, which die at about the four cell stage, as in my group I and the *Nicotiana* × *Petunia* crosses.

4. Crosses in which the true hybrid makes, say, one fourth or more of its normal number of cell divisions, although possibly without any appreciable growth in size, as in my group II.

5. Crosses which result in completely formed hybrid embryos unable in most cases to germinate. Many crosses resulting in non-viable seeds have been reported (51 in my own tests) but in most of such cases the embryo development was not studied. In some instances, however, 1 to 3 percent of the seed has germinated, indicating that embryo growth was practically completed.

6. Crosses like my group III which make a fair to good germination but always die in the seedling stage. Doctor EAST has unpublished observations which show that in some of these there is an accumulation of starch in the cells of the growing point, which the plant is, for some reason, unable to utilize.

7. Crosses resulting in dwarf plants which remain small but in a sense mature and flower.

8. Crosses which produce vigorous, healthy, sterile plants. My *N. paniculata* × *N. glauca* is a case in point.

9. Crosses which produce fertile, or partially fertile, hybrids.

## SUMMARY AND CONCLUSIONS

1. Eight crosses were made, two in each of four different categories based on the success of the hybrid. The resulting hybrid embryos were sectioned and measured at two-day intervals from 7 to 21 days, and growth curves drawn from the data.

2. Fertilization was found to take place usually on the seventh day. There were three exceptions. In *N. rustica* it was earlier as the 7 day embryos had about 32 cells. In *N. Tabacum* × *N. glauca* and *N. rustica* × *N. Rusbyi* no embryos were found until nine days after pollination.

3. The complete development of the *Nicotiana* embryo from the fertilized egg is described and illustrated. This development is similar to that found in other dicotyledonous Angiosperms.

4. Two hybrids, *N. rustica* × *N. glauca* and *N. rustica* × *N. longiflora*, of group I, completed only one or two cell divisions and did not grow at all. They lived only a day or two.

5. *N. rustica* × *N. Rusbyi* and *N. rustica* × *N. Palmeri*, representing group II, experienced a number of cell divisions so that 16 to 32 cell stages appeared but failed to make any appreciable growth.

6. With certain irregularities, which are discussed but for which no explanation is found, the curves of all the other crosses are similar and resemble the familiar logistic, S-shaped curve which has been used to depict the growth of many forms of life.

7. The embryos of group III hybrids, which give a partial germination but die soon after, attain their full size as well as those of group IV.

8. No real difference was noted between the growth of embryos of hybrids of group III, *N. nudicaulis* × *N. Tabacum* and *N. suaveolens* × *N. Tabacum*, and that of embryos of hybrids of group IV. In each case the hybrid embryo curve simulated that of the maternal parent but always fell somewhat below it, even in the most successful crosses, *N. Tabacum* × *N. glauca* and *N. paniculata* × *N. glauca*.

9. Contrary to the findings of BRIEGER who lists only one stage in embryonic development where the hybrid fails, namely, at the time of differentiation of the growing point, I have three groups of hybrids which fail at different stages during embryonic growth or only shortly beyond it.

10. The cause of the failure of hybrids between incompatible species, that is, the nature of the incompatibility, is not studied, but it is pointed out that the observations made lend no support to the hypothesis that it is due to immunological reactions between mother plant and hybrid embryo. The failure of some of the hybrids to make any growth at all suggests rather some inherent physiological inability, dependent upon the unfavorable hybrid genotype.

11. All interspecies crosses are classified in 9 categories, based on the results obtained, ranging from no stimulation in the ovary—complete failure—to the production of fertile or partially fertile hybrids.

#### ACKNOWLEDGMENT

The writer takes pleasure in acknowledging his indebtedness to Doctor E. M. EAST for plants and other equipment provided, and especially for his help in planning this study and his invaluable guidance and advice throughout the progress of it.

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# MUTANTS AND CROSSING OVER IN THE DOT-LIKE CHROMOSOME OF *DROSOPHILA VIRILIS*

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In the oögonial metaphase of *Drosophila virilis*, we find five pairs of the rod-shaped chromosomes similar in length and one pair of the dot-like chromosomes. Hence, in this species, there should be six linkage groups besides the Y chromosome linkage, and one of them is expected to contain fewer genes and to have very low frequency of crossing over.

In the course of genetical investigations with the Japanese stock of *Drosophila virilis*, we have discovered a considerable number of genes which are assigned to six linkage groups (CHINO 1929, 1930 and unpublished data). Of the approximately one hundred genes (the number of genes including multiple allelomorphs in the six linkage groups of *D. virilis*: (X) 35, (II) 19, (III) 11, (IV) 13, (V) 17, (VI) 6) thus far found, the following six are to be assigned to the sixth linkage group in our map.

(1) plain ( $p_1$ ): (March, 1929, CHINO) Recessive, somewhat yellowish body color; dark streak on thorax indistinct; this stock was lost before other genes were discovered.

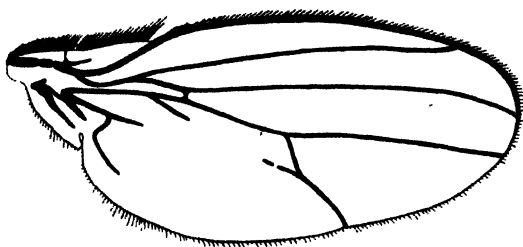


FIGURE 1.—Gap wing of *Drosophila virilis*.

(2) Gap ( $G_p$ ): (January, 1931, CHINO) Dominant, lethal when homozygous; fifth longitudinal vein almost obliterated between the two crossveins; veins near anterior crossvein somewhat disarranged (figure 1).

(3) stubby ( $s_1$ ): (April, 1931, CHINO) Recessive; bristles on thorax and scutellum rather shortened, but not attenuated.

(4) stubby-2 ( $s_2$ ): (April, 1931, CHINO) Recessive; only posterior scutellars shortened and distorted, allelomorph of stubby.

(5) lethal-VIa ( $lv_{VIa}$ ): (February, 1932, CHINO) Discovered in the stubby stock; this makes balanced lethal with the Gap gene.

(6) glossy ( $g_1$ ): (discovered by American authors) Ommatidia irregular; surface of eyes with oily lustre; eye color reddish. (The material with this

character was originally sent us by Doctor M. DEMEREC. After a linkage test, it has been found that this character is to be assigned to the VI chromosome in our map, though it had been located on the IV chromosome in the American map by METZ, MOSES and MASON [1923].)

Besides these six genes, METZ (1923) and DEMEREC (unpublished data) have reported several genes which belong to their sixth chromosome, namely Net, Minute, bent and Pale. But, as the results given later indicate, the genes which are assigned to the sixth linkage group in our map undoubtedly belong to the small chromosome, so that it seems to be desirable to change the original nomenclature published by METZ, etc., and call their IV linkage group the VI linkage group, if it is proper to name the small chromosome the VI chromosome, as METZ and his associates seem to consider (METZ, MOSES and MASON 1923, pp. 77-78).

#### CROSSING OVER EXPERIMENTS

Owing to the dominant character of the Gap gene and the phenotypic clearness with which all the genes express themselves, crossing over experiments on this chromosome can be readily performed. The result of our first experiment to combine Gap with glossy is given in table 1.

TABLE 1  
*Progeny of Gap/glossy × glossy (July 1931).*

NUMBER OF CULTURE	GAP	GLOSSY	GAP, GLOSSY	+	TOTAL
1941	146	145	2	4	297
1942	140	144	4	3	291
1943	120	149	0	0	269
1944	157	163	2	0	322
1945	147	151	1	2	301
1946	139	161	0	1	301
Total	849	913	9	10	1781

Crossing over, 1.06 percent

As this table shows, the two parental combinations in the non-crossover class are approximately equal as are the two new combinations in the crossover class. The frequency of crossing over between these two genes is only 1.1 percent. From these facts, we may infer that crossing over has actually occurred in this chromosome, even though the frequency is very small.

From culture No. 1942 we were able to make one stock combining Gap and glossy in the same chromosome. The result of the unsuccessful second experiment to combine this stock with stubby is given in table 2.

Here, if the frequency of crossing over were about 1.1 percent as pre-

TABLE 2  
*Progeny of Gap, glossy/stubby* × *glossy* (October 1931).

NUMBER OF CULTURE	GAP, GLOSSY	+	GLOSSY	GAP	TOTAL
2002	134	142	0	0	276
2003	124	133	0	0	257
2004	153	128	0	0	281
Total	411	403	0	0	814

Crossing over, 0.0 percent

viously observed, about eight crossovers would be expected among the total of 814 flies. As the cause of the discrepancy between the results of these two experiments the following possibilities may be considered: (a) the genic difference between the stocks used in these experiments, (the frequency of crossing over varies in *D. virilis* according to the strain used); (b) the possible difference in the frequency of crossing over between the cases of coupling and repulsion; and (c) the variation due to external influence, such as temperature, since the first experiment (table 1) was carried out during the summer, while the second (table 2) was performed during the autumn.

In order to solve the above question, we performed the following experiment. Larvae having  $s_l$ ,  $G_p/g_l$  constitution derived from the same

TABLE 3  
*Progeny of Gap, stubby/glossy* × *glossy*, at 30° C and 25° C.

TEMPERATURE	GAP	GLOSSY	GAP, GLOSSY	+	TOTAL
30° C	95	82	0	0	177
	67	81	0	0	148
	47	74	1	0	122
	49	55	0	0	104
	64	75	1	0	140
	82	76	1	0	159
Total	404	443	3	0	850
25° C	82	90	0	0	172
	76	105	0	0	181
	94	92	0	0	186
	91	100	0	0	191
	68	67	0	0	135
	83	109	0	0	192
Total	494	563	0	0	1057

Crossing over at 30° C, 0.34 percent

Crossing over at 25° C, 0.00 percent



parent were divided into two groups and they were bred at 30°C and 25°C respectively. After their emergence, only the females showing Gap character were selected and mated to glossy males, and were bred, as before, at 30°C and 25°C respectively. The results are given in table 3.

As this table shows, each of the three cultures among the six at 30°C gave rise to one crossover individual, whereas no such crossover was found among the six cultures at 25°C, in spite of the greater total number of flies than in the former case. It is, therefore, very likely that high temperature raises the frequency of crossing over between these two genes, even if we admit the other two possibilities, especially the one mentioned under (a). Further details on these points are left for a future study.

The same relation seems to hold also for the region between Gap and stubby, although we have not yet made any detailed study of this matter. Several experiments completed thus far have given the following result in the case of  $G_p/s_l \times s_l$  matings: Gap, 1228; stubby, 952; Gap, stubby, 3; wild, 6. The frequency of crossing over here is 0.41 percent.

The locus of lethal-VIa has not been found as yet, but, judging from the fact that it makes a balanced lethal with Gap, it can be assumed that the gene is located near the three genes described before.

Thus both the data concerning the number of mutants and that concerning the frequency of crossing over suggest that those genes belong to the small chromosome in this species and form the sixth linkage group. This conclusion is strongly supported also by the fact that the crossing over in our IV linkage group is high. For instance, the percentage of recombination between plexus and dachsous is 46.3, between plexus and irregular is 53.5, and that between dachsous and irregular is 22.1 (CHINO 1929, 1930 and unpublished data). This proves that the IV linkage group in our map belongs to one of the longer chromosomes.

#### HAPLO VI IN *Drosophila virilis*

KIKKAWA, one of the authors, found a female (December 1931) with glossy eyes among the  $F_1$  progeny of glossy  $\times$  wild mating. This fly was about  $\frac{2}{3}$  of the normal fly in body size, and had small, slender bristles, blunt and slightly spread wings, and looked weaker than normal. This fly represents probably haplo VI or diminished in *D. virilis*, or the deficiency at the glossy locus. Unfortunately it was sterile.

#### DISCUSSION

In the fourth linkage group in *D. melanogaster*, the following six characters have hitherto been reported: bent, shaven, eyeless, Minute IV, dominant eyeless and rotated abdomen (MORGAN, BRIDGES, STURTEVANT 1925, 1926, MULLER 1930, PATTERSON and MULLER 1930, BELIAJEFF

1931). But, in *D. virilis*, no similar mutant has been found except bent previously reported by METZ, etc. (Very recently CHINO discovered a mutant, rotated abdomen, which seems to correspond to the similar mutant in the IV chromosome of *D. melanogaster*. After a linkage test, it was found that the mutant belongs to the VI chromosome in our map. However, no certain case of crossing over between it and Gap has been found as yet).

Lately, BOLEN (1931) found in the study of X-IV translocations in *D. melanogaster* that the eyeless gene is located at a point distal of the bent gene. However, before this discovery of BOLEN, it has been assumed that the orders of these genes can not be determined from crosses since the crossing over does not occur in this chromosome, and that the cases of apparent crossovers previously reported were probably due to non-disjunction or phenotypic overlapping of genotypic classes (MORGAN, STURTEVANT, BRIDGES 1926, PATTERSON and MULLER 1930).

But, in the dot-like chromosome of *D. virilis*, as our experimental result has proved, crossing over does actually occur, although there is suspicion that some of the cases given before are nothing but the result of non-disjunction. If the glossy and the stubby genes can be put into the same chromosome, we shall be able to decide the orders of arrangement and also the relative distance of these two recessive genes from the Gap gene by a crossing over test. Such an attempt is now underway.

The discrepancy noted between the two species of *Drosophila* concerning the crossing over in the dot-like chromosome may be accounted for to some extent as follows. The X chromosomes seen under the microscope do not show much difference, either in shape or length, between the two species; the genetical maps of this chromosome, however, show a rather considerable difference, comprising about 182 units in *D. virilis* (CHINO 1929), but only 66 units in *D. melanogaster*, (MORGAN, BRIDGES and SCHULTZ 1931, the ratio being 2.8 to 1.

A similar relation is found between the autosomes in the two species. Thus, it may be assumed that crossing over on the whole occurs more frequently in *D. virilis* than in *D. melanogaster*. If so, it is not very surprising if a few cases of crossing over should be found in the dot-like chromosome of the former species.

This fact seems to justify the view that every species has an *a priori* frequency value of crossing over as a whole which is independent of the length of the chromosomes. As a matter of fact, TANAKA (1925) has reported a case in the silkworm where the frequency of recombination exceeds 45 percent notwithstanding the oval shape of all the chromosomes, and HUXLEY (1923) advances a similar view in his work on *Gammarus chevreuxi* where the chromosomes have similar shape.

## SUMMARY

1. Six factors, located in the dot-like chromosome of *D. virilis*, are described: plain, Gap, stubby, stubby-2, lethal-VIa and glossy.

2. Crossing over was found to occur between Gap and glossy, and also between Gap and stubby, the frequency being about 0-1 percent in both cases. The frequency seems to be increased by high temperature.

3. One female probably lacking one dot-like chromosome (haplo VI) was described.

4. A plausible explanation is given for the question as to why the crossing over is found in the dot-like chromosome of *D. virilis*, while it is not found in the similar chromosome of *D. melanogaster*.

## ACKNOWLEDGMENT

In conclusion the authors wish to express their deep obligations to Professor Doctor TAKU KOMAI for his valuable advice and the trouble he took to look through this manuscript. To Doctor M. DEMEREC, also, we are indebted for valuable criticism.

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# CUBITUS INTERRUPTUS, A NEW GENOVARIATION OF THE FOURTH CHROMOSOME OF *DROSOPHILA* *MELANOGASTER*

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The new genovariation here described was discovered in the Normal Florida line in February, 1930, simultaneously at the INSTITUTE OF EXPERIMENTAL BIOLOGY in Moscow and at the CENTRAL GENETICS STATION of Narcomzem (Department of Agriculture). At the CENTRAL GENETICS STATION, the gene-variation was named *cubitus interruptus* ( $c_i$ ) and it was called *cubitus incompletus* in the genetics laboratory of the INSTITUTE OF EXPERIMENTAL BIOLOGY.<sup>1</sup>



FIGURE 1.

## PHENOTYPE OF THE GENOVARIATION

The mutant character consists of one or of several interruptions of the fourth-longitudinal vein (cubitus). Sometimes, additional interruptions are observed in the fifth-longitudinal vein and in the posterior cross vein, which, at times, is even entirely lacking. Sometimes also, the cubitus and

<sup>1</sup> The authors are indebted to Pros. H. K. Koltzoff, Director of the Institute of Experimental Biology for numerous helpful suggestions.

the second cross vein are crooked, and frequently the cubitus is thickened and ragged at the basis. The drawings of six wings in figure 1 give a clearer demonstration of the comparatively strong invariability in the expression of this character. The manifestation of the character in the lines obtained at the CENTRAL GENETICS STATION was complete (100 percent). In the lines of the INSTITUTE OF EXPERIMENTAL BIOLOGY there were inbred cultures where the average manifestation of the character was 98 percent. It is necessary to note that in some lines the percentage of the character manifestation was considerably lower, this being evidently due to the difference of the genotypical medium. The character is very often asymmetrical in its expression, and is sometimes lacking on one of the wings.

#### GENETIC ANALYSIS

In  $F_1$  from crosses of the new mutation with normal lines (Florida and Caucasus Gelendzhik) exclusively normal individuals, in males as well as in females, were obtained (see table 1). Hence it follows that the new genovariation is recessive and not sex-linked. In  $F_2$  a ratio very near to 3:1 was obtained (see table 1).

TABLE 1

CROSSES	$F_1$	$F_2$
♀ $c_i$ × ♂ Normal Florida	1,302 Normal	2,317 Normal: 641 $c_i$
♀ Normal Florida × ♂ $c_i$	365 Normal	(3.13:0.87)
♀ $c_i$ × ♂ Caucasus Normal	353 Normal	2,557 Normal: 786 $c_i$
♀ Normal Caucasus × ♂ $c_i$	185 Normal	(3.06:0.94)
Total	2,205 Normal	4,874 Normal: 1,427 $c_i$ (3.09:0.91)

For determining in which of the autosomes the  $c_i$  gene is situated, the following dominant mutations were used as indicators: for the second chromosome, Lobc 2 ( $L^2$ ) and Star ( $S$ ), and for the third, Dichaete ( $D$ ). As a result of the backcrosses of the  $F_1$  heterozygotes with the double recessives, the data presented on tables 2 and 3 were obtained. In these data the relative lowness of the numbers of  $c_i$  and the relative highness of the corresponding not- $c_i$  class (for example, 556 > 363) is probably due to strong overlapping of the  $c_i$  character with the not- $c_i$  phenotype. This

TABLE 2  
Male backcrosses for chromosome II.

$c_i$	$L^2$	$c_i L^2$	NORMAL
363	595	387	556
$c_i$	$S$	$c_i S$	Normal
99	203	157	217

TABLE 3  
*Male backcrosses for chromosome III.*

$c_i$	D	$c_i$ D	NORMAL
337	307	296	505

suggests that the stocks in which the expression is 100 percent or 98 percent have modifying genes which strengthen the manifestation.

It is seen from these tables that the flies obtained in the male backcrosses were of 4 phenotypes, which shows that no linkage exists either with the second or with the third chromosome characters. One supposition remained possible, that of the gene  $c_i$  being linked with fourth-chromosome characters. To verify this hypothesis a cross was made between homozygous  $c_i$  flies and homozygous flies of the fourth-chromosome character eyeless (Russian mutant allelomorph). All the flies obtained from this cross (527 in number) were normal, as was to be expected, both the genes being recessive, and the flies of this generation being heterozygous by both genes. In the second generation, a segregation into 3 phenotypes, in the proportion of 2 Normal: 1  $e_y^R$ : 1  $c_i$  was expected, and was realized, as table 4 shows.

TABLE 4  
*F<sub>2</sub> from the cross of  $c_i \times e_y$ .*

NORMAL	$c_i$	$e_y^R$	$e_y^R$ $c_i$
3,220	1,422	1,008	0

If the gene  $c_i$  were not carried in the fourth chromosome, we ought to have received four phenotypes in the ratio of 9 Normal: 3  $c_i$ ; 3  $e_y^R$ : 1  $e_y^R c_i$ . Since no phenotype  $e_y^R c_i$  was obtained the data prove that the  $c_i$  gene is carried in the fourth chromosome. One of the authors of the present paper (G. G. TINIAKOF) has obtained through crossing over in the fourth chromosome between eyeless (Russian) and  $c_i$ , a stock of  $e_y^R c_i$ .

We had the opportunity of demonstrating this stock to BRIDGES on his visit to our Institute in January, 1932, and he confirmed our classification. He also stated that this is the first definite evidence of crossing over in the fourth chromosome. His own experiments had given apparent crossing over (0.9 between eyeless and bent), but some of this apparent crossing over was undoubtedly due to non-disjunction instead and he had not excluded the possibility that all of it was due to non-disjunction and that perhaps the fourth chromosome was too short to give the usual kind of crossing over. With the  $e_y^R c_i$  stock further work is now being carried on for testing the amount of crossing over between the genes in question. The results will be published later.

## SUMMARY

The new character here described is due to a recessive gene in the fourth chromosome in *Drosophila melanogaster*. The gene causes varying breaks and abnormalities in the veins of the wing, principally in the cubitus and occasionally in the analis and in the posterior cross vein. In some lines the genovariation  $c_i$  has a complete manifestation (100 percent) while in other lines it is 98 percent or lower; in the  $F_2$  from out-crosses it may be as low as 75 percent. This probably means that the high manifestation stocks have intensifying modifiers which are separated from  $c_i$  in the segregation. Definite evidence that crossing over occurs in the fourth chromosome between the loci eyeless (Russian) and  $c_i$  was obtained.

# CHIASMA FORMATION IN LARIX AND TSUGA\*

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From the standpoint of chiasma formation and the relation of the chromatids, two essentially different theories have been advanced. The first is JANSSENS' "partial chiasmotypy" hypothesis (1924). This theory enjoins a crossing over between two of the four chromatids, a crossover at every chiasmata, and a pairing of only sister threads at early diplotene. The Janssens' "partial chiasmotypy" theory, with various modifications, is supported by BELLING (1931), DARLINGTON (1931), and MAEDA (1930).

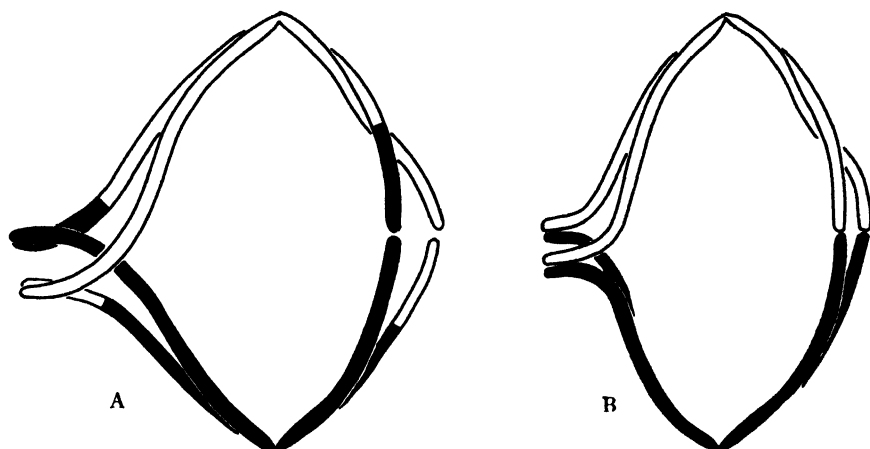
The other hypothesis is that of McCLUNG (1927). The work of WENRICH (1916), ROBERTSON (1916), CAROTHERS (1926, 1931), NEWTON (1927), BELAR (1928), and many others supports this theory. Chiasmata are produced by an alternate pairing of sister and non-sister strands. Chiasmata represent an exchange of partners among the chromatids. According to SAX (1930), a crossover occurs only when two chromatids break at a chiasma. On this theory chiasmata may be present without crossing over.

McCLUNG (1927) has pointed out clearly that when there is crossing over, according to JANSSENS' theory, there should be an asymmetrical arrangement of the chromatids. JANSSENS based his arguments for his theory on cases where the asymmetrical arrangements have been found in certain Orthoptera, but as McCLUNG has pointed out the clearest figures show that symmetrical figures are the usual and most prevalent types. On any theory, crossing over between two of the four chromatids should usually result in an asymmetrical relation of the chromatids. If a single crossover occurs it is possible that the chromatids might be subsequently oriented to produce a symmetrical relation of the chromatids, but in case two crossovers occur in a single bivalent a symmetrical relation of chromatids should be exceptional. Viewed from the end, each of the four chromatids should not lie in the same quadrant at all loci after a crossover has occurred at the four strand stage. As the homologues open out, the chromatids which were adjacent may form the cross at the chiasma if the chiasma is the result of a reciprocal crossover (text figure 1A). As such a chiasma is terminalized there should be a twist in one pair of chromatids. These reciprocal crossovers should occur as frequently as equationals if crossing over is at random. Equational crossovers should also result in asymmetrical relations of the chromatids although it is possible that chro-

\* The cost of accompanying heliotype plate is paid by the GALTON AND MENDEL MEMORIAL FUND.



matid movements in subsequent stages might produce symmetrical arrangements of chromatids in some cases. If each chiasma represents a crossover as is postulated in JANSSENS' "partial chiasmotypy" hypothesis, the bivalent chromosomes with one or more chiasmata should show a prevalence of asymmetrical chromatid relationships.



TEXT FIGURE 1.—A. Chromatids at metaphase stage of meiosis according to the Janssens hypothesis. The arrangement of the chromatids is asymmetrical. The chiasmata represent crossing over. The chiasma at the left is formed by adjacent chromatids. On the right the chiasma has terminalized. There is a twist in the upper pair of chromatids. B. Chiasma formation according to the McClung theory. The cross in the chiasma at the left is formed by diagonal chromatids. At the right the chiasma has terminalized and the asymmetrical arrangement is due to a half twist at early prophase.

On McCLUNG's theory, chiasma formation is not the result of previous crossovers, so that a symmetrical arrangement of chromatids might be expected if there are no crossovers or no twists in sister chromatids. Each chromatid should lie in the same quadrant at all loci at the four strand stage, and as the paired chromatids open out at diplotene and metaphase the apparent cross at the chiasma is formed by chromatids which were diagonal at the four strand stage (text figure 1B). A half twist in sister chromatids before or after pairing of homologous chromosomes would produce an asymmetrical relation of chromatids even if no crossover has occurred (text figure 1B). A crossover resulting from a break in two chromatids at a chiasma (SAX 1932) would result in an asymmetrical relation of chromatids.

A prevalence of symmetrical chiasmata have been shown in Orthopteran chromosomes by WENRICH (1916), ROBERTSON (1916) CAROTHERS (1927) and DARLINGTON (1932). As McCLUNG (1927) has pointed out, JANSSENS' (1924) clearest figures also show a symmetrical relation of chromatids. Both symmetrical and asymmetrical chiasmata have been found in *Paeonia* (SAX 1932b). The symmetrical double chiasmata show clearly

that no crossovers have occurred in these chromosomes and that chiasma formation is not dependent on a previous crossover. Asymmetrical chiasmata would be expected if a crossover has occurred at another chiasma or if a half twist was formed between sister chromatids. In some cases the cross at the chiasma was formed by the two threads which were adjacent at the four strand stage. If, as the work of WENRICH (1916) and CAROTHERS (1931) suggests, sister chromatids are not always associated at the fiber attachment point, these chiasmata might be attributed to an equational separation at the first meiotic division associated with a half twist in sister chromatids.

MAEDA (1930) has observed asymmetrical chiasma formation in *Lathyrus odoratus*. This would be expected as crossing over occurs in *Lathyrus odoratus*, and on any theory of crossing over asymmetrical chiasmata are formed in crossing over.

#### CHIASMATA AND CHROMATIDS IN LARIX AND TSUGA

The present investigation is concerned with chiasma formation and the relation of the chromatids in *Tsuga* and *Larix*. *Tsuga canadensis*, *Larix decidua*, *Larix Kaempferi*, and the hybrid *Larix eurolepis* Henry (*L. Kaempferi* × *L. decidua*) offer very good material for the study of the chromatids.

Permanent smears and paraffin sections of the microspore mother cells of *Larix* and *Tsuga* were made. The material was fixed in Navaschin's, La Cours', and Darlington's modification of Flemming's stronger fixatives. Permanent smears with the latter fixative gave the best results. Crystal violet iodine gave the best results in staining the chromosomes. Flemming's triple stain was a better stain for the fibers.

The divisions in the microspore mother cells of *Tsuga canadensis* are especially favorable for the study of chiasmata. The term "chiasma" will be used here to designate the region where there is an exchange of partners among the chromatids. The chiasmata are very similar to those found in *Larix* (SAX 1932, plate 51), although the chromosomes are somewhat larger. During diakinesis the configurations of chromosomes and the number and position of chiasmata can be observed. The prevalence of interstitial chiasmata suggests that there is very little terminalization before the metaphase stage.

Figure 1, plate 1, shows all the chromosomes drawn from a side view of a late metaphase stage in the first division of a microspore mother cell of *Tsuga canadensis*. They are drawn separately to show the detail of each one. The number and position of the chiasmata can be clearly seen in some of the chromosomes, while in others some of the chiasmata have opened out because of the lateness of the stage. This point is easily inferred

## LEGEND FOR PLATE 1

All drawings were made from the first division of the microspore mother cells, with the aid of a camera lucida. The magnification is  $\times 3100$ .

FIGURE 1.—Twelve chromosomes, a, b, c, d, e, f, g, h, i, j, k, and l are drawn from a single late metaphase stage in the first division of the microspore mother cell of *Tsuga canadensis*. The number and type of chiasmata are shown.

FIGURES 2 and 3.—Bivalents of *Tsuga canadensis*.

FIGURE 4.—Bivalent from *Larix eurolepis* at diakinesis stage with two interstitial chiasmata.

FIGURE 5.—Chromosome from *L. eurolepis* in late diakinesis. Two chiasmata, one subterminal, the other interstitial are present. The chromatids are separated in the homologues.

FIGURE 6.—Chromosome—late metaphase in *L. eurolepis*. Chromatids symmetrical.

FIGURE 7.—Chromosome at late metaphase in *L. eurolepis*. Chromosome shows stretching between chiasma and fiber attachment.

FIGURE 8.—Bivalent, late metaphase in *L. eurolepis*. One interstitial chiasma. Symmetrical arrangement of chromatids.

FIGURE 9.—Bivalent, late metaphase in *L. Kämpferi*. Two subterminal chiasmata.

FIGURES 10 and 11.—Metaphases of bivalents of *L. eurolepis* showing symmetrical arrangement of chromatids widely separated with two interstitial chiasmata.

FIGURE 12.—Metaphase of chromosome of *L. eurolepis* with chromatids separated on left side. Symmetrical arrangement of chromatids.

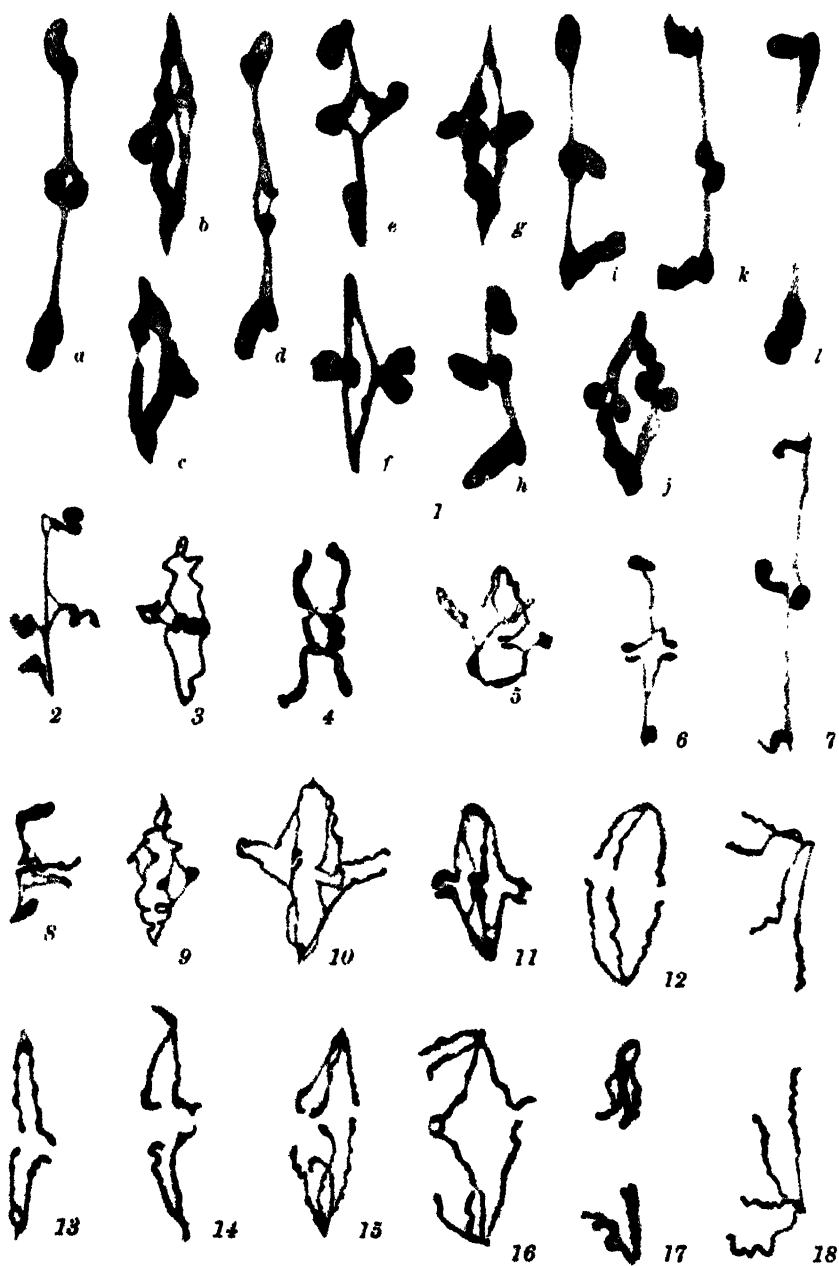
FIGURES 13 and 14.—Chromosomes with fiber attachment, terminal or subterminal. Chromatids separated. Chromatids symmetrical.

FIGURE 15.—Chromosome, *L. eurolepis*, early anaphase. Asymmetrical arrangement of chromatids.

FIGURE 16.—Chromosome in late metaphase, *L. eurolepis*. One chromatid from each homologue separated, the remaining chromatids still paired. Symmetrical arrangement of chromatids.

FIGURE 17.—Anaphase stage of bivalent, *L. eurolepis*. Asymmetrical arrangement.

FIGURE 18.—The same, showing symmetrical arrangement.





from the large number of stages at diakinesis and metaphase where the chromosomes have not started to divide. There are several chromosomes (figure 1a, d, h, i and k), where the homologues have opened out at one end. In figure 1l, the homologues have completely separated. There are interstitial and subterminal chiasmata which are very distinct in most of the chromosomes shown in figure 1. Some of the chromosomes show an opening where the chromatids change partners. There is considerable strain on the homologues between the chiasma and the point of fiber attachment. They are very narrow in this region. This is shown in *Tsuga* (figure 1a, d, i, and k) and in *Larix* (figure 7).

With very good fixation and lighter stain only the chromatids are seen. The relations of the chromatids at the chiasmata are somewhat more distinct. Figures 2 and 3 from *Tsuga* show a twisting of the free ends of the chromatids near the chiasmata.

The chromosomes in the divisions of the microspore mother cells of *Larix* were especially favorable for the study of the chiasmata and the relations of the chromatids in the bivalents. In the hybrid *Larix eurolepis* the pairing between the chromatids appears to be a little less close, and, in some cases, the individual chromatids are distinctly separated. Though perhaps not so distinct, the chromatids are found to have a similar arrangement in the parents, *L. Kaempferi* and *L. decidua*. In most cases, however, in both parents and hybrids the pairing was too close to follow the chromatids throughout, but the chiasmata are quite distinct and the chromosomes very similar in the hybrids and parents. However, a large number of diakinesis, metaphase, and anaphase figures were found in the hybrid in which the chromatids were distinct, and from these most of the drawings were made.

Figure 4 is drawn from a late diakinesis stage. There are two interstitial chiasmata. The homologues are widely separated between the chiasmata and at the free ends beyond the chiasmata. The chromatids are not separated in this case.

Figure 5 represents a late diakinesis, or very early metaphase stage. The chromatids in the two homologues of the bivalent are separated from each other so that they may be clearly seen. There may be a twisting in the chromatids in this case in the lower half. One chiasma is median between the fiber attachment and the end of the chromosome; the other is subterminal. The homologues are again widely separated except at the chiasmata.

In figure 7, a late metaphase, there is an interstitial chiasma with a distinct opening where the chromatids exchange partners, a slight coiling of the free ends of the chromosomes beyond the chiasma, and a stretching of the chromosome between the chiasma and the fiber attachment, indicating some strain in the separation of the paired chromatids.

In figure 6, which is a very late metaphase, the chiasma is subterminal. The paired chromatids are distinct and are separating at the chiasma. The arrangement of the chromatids is symmetrical.

The details in the separation of the paired chromatids in an interstitial chiasma are easily seen in figure 8. If one imagines the uppermost pair of chromatids rotated from the plane of the plate through the vertical position and to a position making an angle of  $180^\circ$  with the present position, one will recognize that the arrangement of the chromatids in this case is similar to that in figure 6; but the chiasma is interstitial. The arrangement of the chromatids in figure 8 is obviously symmetrical. The diagonal chromatids form the cross. In other words, the union of the homologues here is face to face, and not at an angle and lateral.

Two chiasmata, both subterminal, with a comparatively wide space between the chromatids, are seen in figure 9, which was drawn from *L. Kaempferi*. The arms of the bivalent between the fiber attachment and the chiasma show some coiling.

In figure 10, the chromatids are widely separated so that, except at the point of fiber attachment, they are very distinct and easy to follow. The chiasmata are interstitial. The chromatids from the upper homologue pair separately with those from the lower homologue. Two of the pairs of chromatids are touching at the ends. The other two are paired, but they are not in contact in any place. The chromatids are symmetrical on both sides. No asymmetrical arrangement is possible with such wide separation of the chromatids. If the cross had been made by adjacent chromatids, they would not be free to separate so widely in interstitial chiasmata.

Figure 11 is similar to figure 10, with somewhat thicker chromatids. The chromatids to the left have a connection near the distal end, which may be due to a twisting about each other. It is obvious, however, that the arrangement of the chromatids is symmetrical, since the chromatids of the homologues are separated beyond the region of the chiasmata. On the left side of the bivalent shown in figure 12, the chromatids are symmetrical. They are separated throughout their length.

In figures 13 and 14, the symmetrical arrangement of the chromatids is apparent. The spindle-fiber attachment is near the end of the chromosome. The paired chromatids are separated, and the ends of the homologues are separating.

In figure 15, the chromatids are all distinct, and the ends, where the homologues have paired, are separating. There is an appearance of an asymmetrical arrangement in this case.

A slightly more advanced stage is found in figure 16. The symmetrical arrangement of the chromatids is the only one possible in this case. The fiber attachment is in the middle of the homologues of a bivalent with two chiasmata. The chromatids are very distinct. One chromatid on each side

has separated completely at the region of pairing with its homologue, and has its free end in the position characteristic of the anaphase. The other two chromatids of the homologues were still paired at the ends. The arrangement of the chromatids is obviously symmetrical. An asymmetrical arrangement of the chromatids is impossible in this case.

The arrangement is asymmetrical in figure 17. In this case there is a distinct twist in the chromatids in the upper half of the figure.

Figure 18 is an anaphase. The configuration of the chromatids appears to be symmetrical. The chromatids are distinct. They are held together only at the spindle fiber attachment. In the lower half of the figure, they are loosely associated at the region of the spindle fiber attachment point.

#### DISCUSSION

The figures described above show a great preponderance of symmetrical figures. Though a thorough search was made among the figures of *Larix* showing separate chromatids, to find chromosomes where the arrangement of the chromatids was obviously asymmetrical, only the few possible cases mentioned above were found. There was manifest almost a total uniformity of symmetrical configurations in the chromatids of *Larix*.

The greater frequency in the occurrence of symmetrical configurations in *Larix* supports the McClung theory. They could not occur if the chromatids had paired according to the Janssens hypothesis.

Very few cases were found where an asymmetrical arrangement of chromatids is found in *Larix*. It is possible in some figures, where a symmetrical arrangement is found, that an asymmetrical arrangement existed earlier and that through a readjustment in the chromatids this asymmetry was straightened out. This may occur where the spindle fiber attachment is near the end of the chromosome. Figures 13 and 14 represent chromosomes of this type. The configurations are obviously symmetrical.

However, a large number of bivalent chromosomes with two chiasmata were found where there was no possibility of an asymmetrical arrangement of the chromatids, which would be expected on the Janssens hypothesis. This arrangement is in agreement with the McClung theory. According to SAX, some cases of asymmetry would be expected on the McClung theory if there was a half twist in sister chromatids before pairing in the early prophase, or if crossing over had taken place.

SAX (1932) found some cases in *Paeonia* where adjacent chromatids form the cross at the chiasma. This may seem to support JANSSENS' "partial chiasmotypy" theory. These cases may be explained on the McClung theory if non-sister threads are held together at the fiber attachment associated with a half twist in one pair of sister chromatids. From the behavior of heteromorphic chromosomes in Orthoptera, according to WENRICH and CAROTHERS, an equational first meiotic division seems to be possible.



The preponderance of the symmetrical arrangement of the chromatids in *Larix* suggests that there is little crossing over in *Larix*, even though the chiasma frequency is relatively high. Previous investigations have shown that the rarity of polyploidy in the conifers may be attributed in part at least to the high chiasma frequency (SAX 1932). The symmetrical chiasmata indicate that little or no crossing over occurs in this group of plants. These two factors, uniformity in chromosome numbers and absence of crossing over between chromosomes, may be of importance in explaining the relative stability of the conifers. In the genus *Larix*, distinct species which have been geographically isolated for long periods of time produce fertile hybrids, and in general the genera of conifers are not highly polymorphic.

## SUMMARY

The divisions in the microspore mother cells of *Larix* and *Tsuga* are especially favorable for the study of the arrangement of chromatids because the chromatids are often seen separated in the homologues. In most cases the chromatids are symmetrical in arrangement, and the cross in the exchange of partners is formed by diagonal chromatids. These configurations seem to be incompatible with the present interpretations of the Janssens hypothesis. In a very few cases there are asymmetrical configurations. This arrangement of chromatids would be expected on the McClung theory if either a half twist of sister chromatids or a crossover had occurred in the prophase. The preponderance of symmetrical figures, together with little terminalization of the chiasmata up to metaphase, would indicate that little crossing over occurs and that chiasma formation may be independent of crossing over.

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# THE INHERITANCE OF ALLERGY WITH SPECIAL REFERENCE TO MIGRAINE<sup>1</sup>

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For some years during the course of treatment of different forms of allergy at the BALLYEAT HAY FEVER AND ASTHMA CLINIC in Oklahoma City, various facts concerning the inheritance of allergy have been noted. It is the object of the present study to determine as far as possible from the material thus collected the type of inheritance displayed by that form of allergy called migraine. The study was made entirely from a genetic point of view and the material used began with the available records of patients of the BALLYEAT HAY FEVER AND ASTHMA CLINIC.

Studies on the heredity of allergy are not numerous. Work seems to have begun about 1909 with DRINKWATER's results which indicate that asthma acts as a dominant character. Since that time a number of investigators have written on some phase of allergy. Some, though reporting on one form of allergy, have realized that various others have occurred in one family. A connection of migraine with the sex endocrines has been observed, several investigators noting that affected females suffer with migraine more frequently during the menstrual period.

ADKINSON has made an extensive study of bronchial asthma (1920) and she concluded that it is a recessive character, since normal people transmit the asthmatic tendency. These normals she regarded as heterozygous. Her findings do not all bear out this conclusion, for she states that children of two heterozygous normals are three times as likely to be asthmatic as normal, whereas one fourth should be asthmatic if the character is a true recessive. She seems to have overlooked the possibility of incomplete dominance. ADKINSON found that bronchial asthma, hay fever, some urticaria, and eczema must be accepted as equivalent in their inheritance. She states that "asthma is inherited with equal frequency from the family of either parent."

BUCHANAN in 1920 considered migraine as a recessive trait. SMITH (1922) concluded that migraine is a dominant trait, in some way sex determined. The work of STUDY, referred to by BAUR, FISCHER, LENZ, indicated that hay fever is a dominant character, manifesting itself in the older members of a family as gout, but in the younger as hay fever. GÄNSSLEN (1921)

<sup>1</sup> Studies from the Zoological Laboratory of the University of Oklahoma, Second Series No. 114.

showed that one individual, and sometimes a number in the same family, often are subject to hay fever, asthma, urticaria, and eczema, gout and gall stones.

LENZ (in BAUR, FISCHER, LENZ), using the charts of UNGER and WIETZ, concluded that migraine is probably a sex-linked dominant and that "the sexual life seems to have an influence upon the occurrence of migraine. There are women who suffer from migraine only when they are menstruating, and never have an attack during pregnancy."

FETSCHER (1927) suggested that hay fever is sex-linked. He found that sons only were affected if both parents were negative and daughters were affected if one parent was allergic. Since he did not prove that hay fever is transmitted to sons from their mother only, and since he showed only three charts, his results are not conclusive.

ALLEN (1927-28) analyzed 400 cases of migraine and concluded that it is inherited as a dominant character. He found that menstruation influenced the onset of migraine. JORDAN (1928) published a family record to show that eczema is inherited.

In 1928, BALLYEAT showed that inheritance appears to be the chief factor in determining hay fever and asthma and that the earlier in life an individual becomes sensitive, the greater the tendency to develop a sensitivity to more than one group of proteins. He found that the environment to which an individual is exposed has much to do with the sensitivity which develops. Also he stated that eczema and migraine are interchangeable with hay fever and asthma, and that the thing inherited is the ability to become sensitive, but not the specific state, and that this is inherited as a dominant character.

In 1929, the writers of this paper made a preliminary genetic study of twelve family pedigrees. They concluded that there is probably only one gene involved in the different allergies and that the gene acts as a dominant Mendelian unit, although its dominance is often incomplete, that it sometimes skips a generation, and that the environment is at least to some extent responsible for the expression of this gene.

In 1930, APPERLY published a family tree showing five generations, in each of which allergy occurred. In the same year ELY showed that migraine is an hereditary disease.

In the study of the inheritance of migraine from a genetic point of view it might be of interest to point out some clinical observations. BALLYEAT (1931) in a study of a series of 202 cases of migraine concluded that migraine appears to be common in children since nearly one-third of all cases developed symptoms during the first decade, that the syndrome is more common in business men, professional men and teachers than in laborers, that about 7 percent of all people in the United States some-

time in life suffer from the symptom complex, that not unlike asthma symptoms of migraine in childhood may vary greatly from those of the adult, and that migraine is interchangeable in the linkage with other allergic diseases. In an unpublished paper (RINKEL and BALYEAT 1932) in a series of 65 cases of headache (migraine) proved to be due to food hypersensitiveness, a family history of headache was obtained in 84.4 percent. It appears from our clinical study that a patient transmits from one generation to another only the ability to become sensitive and not the specific state. Apparently the type of sensitivity from which the antecedent suffered has no relation to the type the descendant may have.

In using the term "migraine," we refer to those patients who have paroxysmal headaches characterized by hemicrania (also bilateral headaches), and who have symptomatic evidence of cortical involvement. There are headaches due to hypersensitiveness that cannot be classified as migraine since they do not have cortical features. These cases have not been used in our study. It may be remarked that this restricted definition of migraine makes even more striking some of the genetic situations later described, in which persons who have only migraine transmit several other allergies to their descendants.

The methods used in the present work are similar to those by which material was collected for our preliminary paper. Detailed questionnaires to patients were employed to obtain the family histories used for our pedigrees. The questionnaires asked for specific facts concerning each member of the family. When there was any doubt as to an answer, letters were written about specific points, and in many cases a series of letters was sent to a single individual. Since the patients treated by the clinic are of a high type of intelligence (see below), the information obtained is looked upon as very trustworthy.

We realized at the outset the necessity of getting full pedigrees of both maternal and paternal sides of the families. The work was done as far as possible with methods commonly employed in genetics. This is in contrast with methods sometimes used in studies of this nature in which records are kept only of the affected parents of the individuals being studied, omitting the contribution to the inheritance made by the parent who may not happen to show the character. Of this latter nature are many of the charts referred to by BAUR, FISCHER, LENZ including the pedigrees relating to allergy from GÄNSSLEN, UNGER, CROWDER, ULLMAN, and STUDY.

Data as complete as could possibly be obtained were collected from fifty-five families, both maternal and paternal sides of each pedigree being included. Forty-one of these pedigrees gave a sufficiently complete history for genetic use. We have retained only the pedigrees which gave fairly complete information about two and usually three generations. Most of

the people to whom questionnaires were sent have suffered from migraine. The preliminary report concerning hay fever was based upon a study of twelve families. Including the pedigrees of the earlier paper, therefore, fifty-three out of sixty-seven different family histories complete enough for genetic study form the basis of this report.

In the preliminary report we found that hay fever and other forms of allergy may act as a dominant character but that their dominance is irregular, for it is often incomplete and sometimes an unaffected individual may transmit the ability to become sensitive to several or to all of his or her children. In the earlier paper the various forms of allergy were considered as different expressions of a single gene, although the possibility of multiple factors as a cause was not overlooked. The genetic evidence then available led to this conclusion, but it is to be noted that it was quite in harmony with the clinical behavior of the diseases.

After studying allergic families for several years we have found that if allergy is present in a strain, it is extremely rare (families 2 and 23) for a generation to occur without some member of the family being affected. We have only five cases of this kind. Unaffected individuals may pass on the allergy to their children, but usually these individuals have allergic brothers or sisters even though they themselves have no allergic diseases. In no case which we have studied does allergy skip two generations; that is, allergic individuals have never been found to appear after the family has been negative for two generations. Occasionally one generation may be entirely skipped, but we have no record of two negative generations followed by a third allergic one, and we have sought carefully for cases bearing upon this point. For these reasons we have come to regard a family as incapable of transmitting the gene for sensitivity if in our pedigrees it has been entirely negative for two generations, or if the parental generation and the two grandparents also are negative.

The changing or incomplete dominance of allergy may best be explained by quoting a couple of paragraphs from our earlier paper:

"The time of appearance of the sensitivity in the ontogeny of the individual differs greatly in different people. In some cases a child develops a sensitivity very shortly after birth and in other cases individuals who have never before had trouble develop a sensitivity at the age of seventy. Between these two extremes are a whole series of ages, at any of which a sensitivity may develop. Consequently, if an individual belongs to an affected family and has had no trouble, there is still a great probability that he may later develop it. Thus no chart of an affected family can ever be considered as complete, for those once classed as normal may later develop some form of allergy.

"Another point must also be considered. In order for any form of sensi-

tivity to become manifest there must be, in addition to the sensitivity, the external factor that causes the trouble. A child sensitive to certain forms of protein will not develop urticaria or eczema unless he eats that protein. Similarly an individual sensitive to a certain kind of pollen develops hay fever only when exposed to that pollen. In recent years much has been added to our knowledge of the interaction of heredity and environment. Apparently the study of inheritance of hay fever, etc., reveals another case in which a definite environment as well as a definite inheritance is necessary to produce a definite character."

These statements of our earlier paper apply equally well to migraine. This form of allergy sometimes occurs in young children and on the other hand many people from allergic families develop this difficulty much later in life. The environment necessary to produce allergy in members of an allergic family differs with respect to the different allergies. Hay fever does not develop unless the patient comes in contact with a particular kind of pollen which furnishes the necessary irritation. For example, a patient born with the ability to become sensitive, who comes in contact with Russian thistle pollen develops a sensitivity to Russian thistle and may have hay fever symptoms on adequate contact. Likewise, those who live in the timothy section of the United States and who come in adequate contact with timothy pollen have developed a sensitivity to timothy and have hay fever symptoms from contact with timothy pollen. Similarly migraine, eczema, etc., develop only when the foods to which the individual is sensitive are eaten. Both the pollen in the one case and the particular food in the other may be regarded as the environment necessary to bring out the allergy.

The data upon which these conclusions in regard to migraine are based are less open to criticism than in the case of hay fever on the score of the environmental condition necessary to bring out the sensibility. That is, there is less possibility that the negative cases are not truly negative but have merely not happened to meet the proper environment to cause the allergy to manifest itself. The reason for this is that with the normal varied diet which most adults enjoy there is little chance that a person can escape the sensitizing protein long after reaching adulthood.

That both a definite heredity and a definite environment are necessary to produce a given character is well known and many cases of both animals and plants can be cited to support this principle. The early work of BAUR on the effect of temperature on the color of the primrose was perhaps the first case of this kind noted. Since that time modern work has shown that many adult characteristics are dependent on a special environment as well as on a special gene for their fulfillment. MORGAN'S work on abnormal abdomen in *Drosophila* showed that this character is largely dependent upon moisture. IRA WILSON'S hereditary tumor in *Drosophila* is dependent to a

large extent upon the amount of food. The work of the senior author on extra legs in *Drosophila* (HOGG 1914) has shown that this character appears only when the conditions of temperature are suitable. Similarly KRAFKA has demonstrated that the number of facets of "bar eye" in *Drosophila* varies in relation to temperature. Also there are many examples to show that age may operate to make manifest the determining factor in various characters. Pink-eyed flies have light eyes when young, but when old their eyes are almost as dark as those of the wild red fly. Black-bodied flies when young are as light colored as the normal fly and they develop black pigment only with age. These examples are sufficient to show that

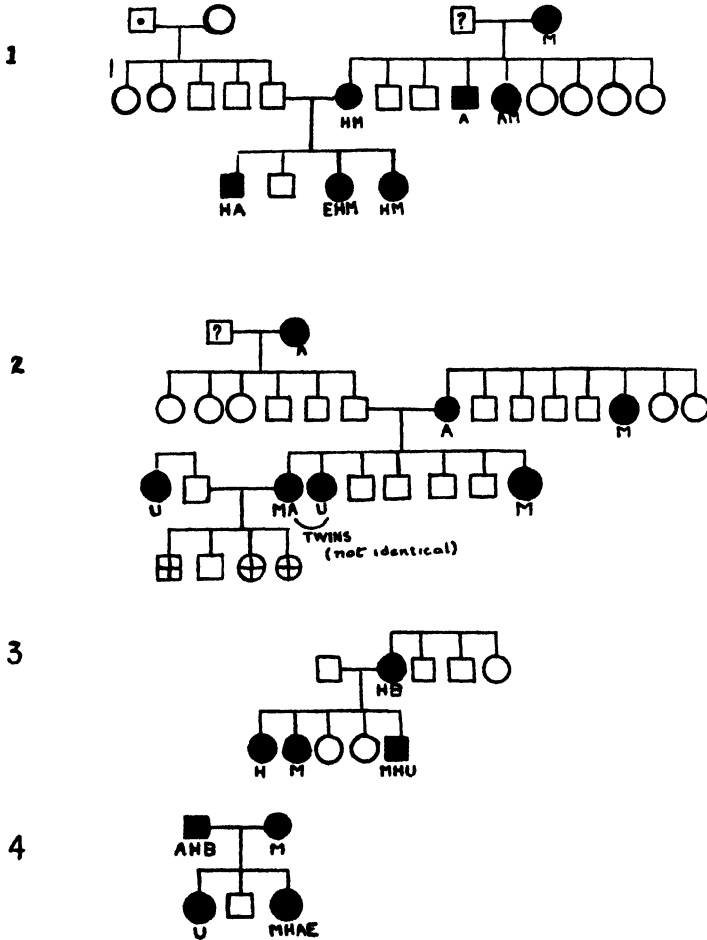
## Key to Charts

○	female	A Asthma
□	male	B bronchitis
● ■	allergic individuals	C colitis
⊙ ⊡	on known facts concerning individual	E eczema
○ □	individuals known to be negative	H hay fever
⊕ ⊡	individuals who died young	M migraine
⊙ ⊡	individual considered negative, but data incomplete	U urticaria
⊗ ⊡	family history incomplete as to members; except in cases so marked, every individual in every family is listed	
● ■	allergic, but type unknown	
⊖ ⊡	individuals from families with no allergy	
○ □	negative individuals from families known to be allergic	

both environment and heredity are determining factors in the development of a particular individual. Studies on the inheritance of allergy show that with it, just as with these cases of the lower animals and plants, an individual does not become allergic unless the environment to which he or she is subjected is such as to bring out the latent sensitivity.

An interesting fact early observed in this study was that allergic persons commonly marry into allergic families. This is still true even in the cases where one or both of the individuals concerned are themselves negative. Our records include only a few cases in which marriage occurs between a person from an allergic family and one from a negative line. The explanation for this fact can only be conjectured. Allergic families have

been found to be usually of a high grade of intelligence, and, as BALYEAT (1929) has shown, a hay fever sufferer customarily has a higher intelligence quotient than does the average person. It may be surmised that this fact has something to do with the frequent marriages between allergic families and that in some cases their higher intelligence serves as a mutual attraction. Since allergic persons make up only a small percentage of the entire

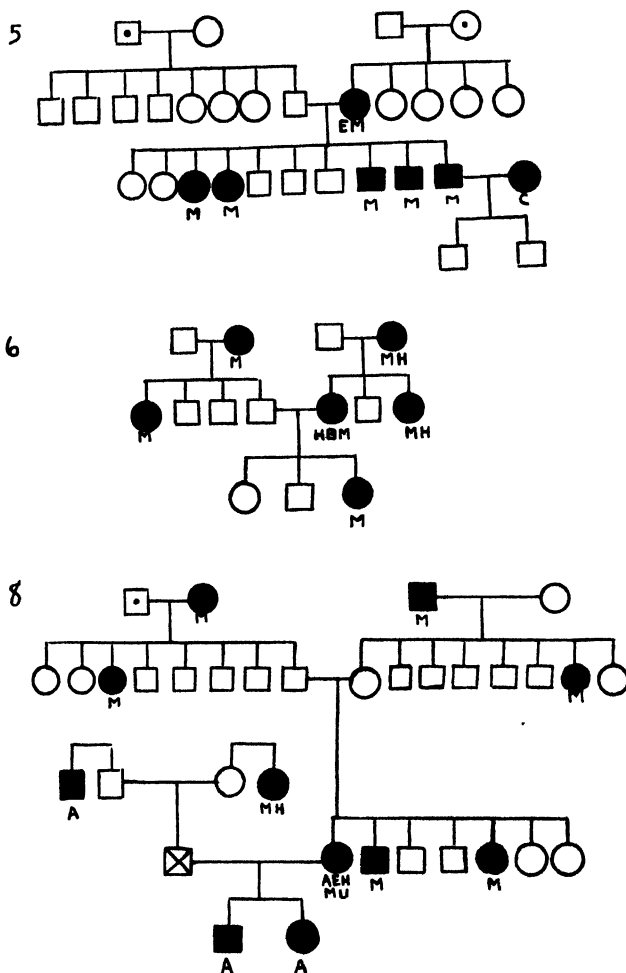


population, their frequent marriage is worthy of note, although no means is at present known which favors the mating of these persons.

In our paper on hay fever we have stated that some families show only one form of allergy. It is seldom, however, that any family is specific for one allergy alone. Only one of the twelve families of our earlier paper showed no allergy but hay fever. One had only asthma and hay fever, and the other ten had in addition either eczema, bronchitis, urticaria, migraine, or a combination of several of these allergies.



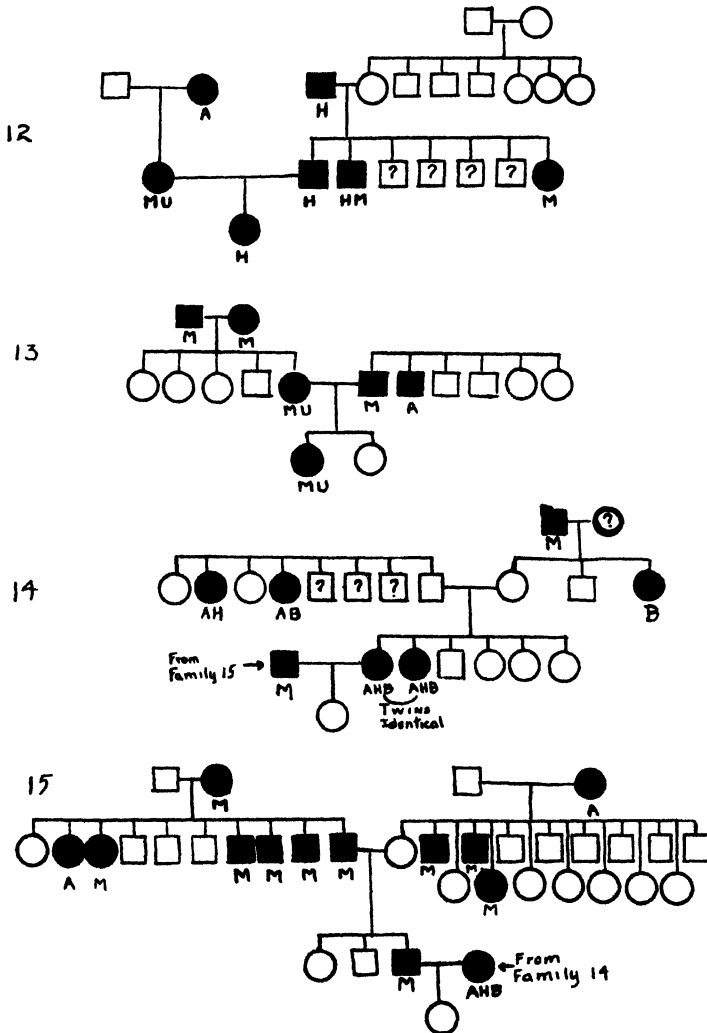
Our present study of forty-one families shows similar conditions. Only five (for example families 22, 23, 27, 37) exhibit branches which before crossing with other allergic families show nothing but migraine, and in most of the families there are various combinations of the different allergies, a single individual sometimes suffering from four to five types (example, family 8). In eleven families there are two kinds of allergy and in twenty-six there are three or more.



Since in the families from whom we have collected data so many forms of allergy are present, and since allergics commonly marry individuals from similar families, it is extremely difficult to determine positively the number of genetic factors that cause the different forms of sensitivity.

In our present study we have endeavored to ascertain whether our pedigrees could be explained as due to the operation of several genes acting

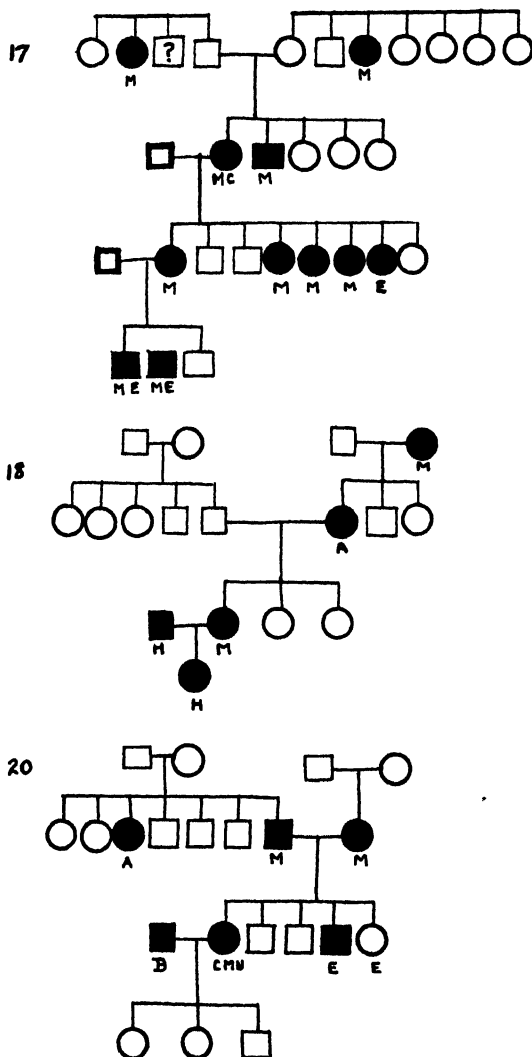
together, perhaps one gene being necessary for an individual to become allergic and a special modifying gene being necessary to produce each type of allergy. This possibility, mentioned in our previous paper, has been suggested by several critics, but the number of pedigrees previously considered was insufficient to settle the point.



In order to gain some light on this matter we have examined carefully the pedigrees of those families in which an allergic individual marries into a negative line. As already stated, a line is considered negative when for two generations no member has exhibited an allergy or when the parental generation and the two grandparents also are negative. The study of this type of mating has proved the most productive phase of our research.

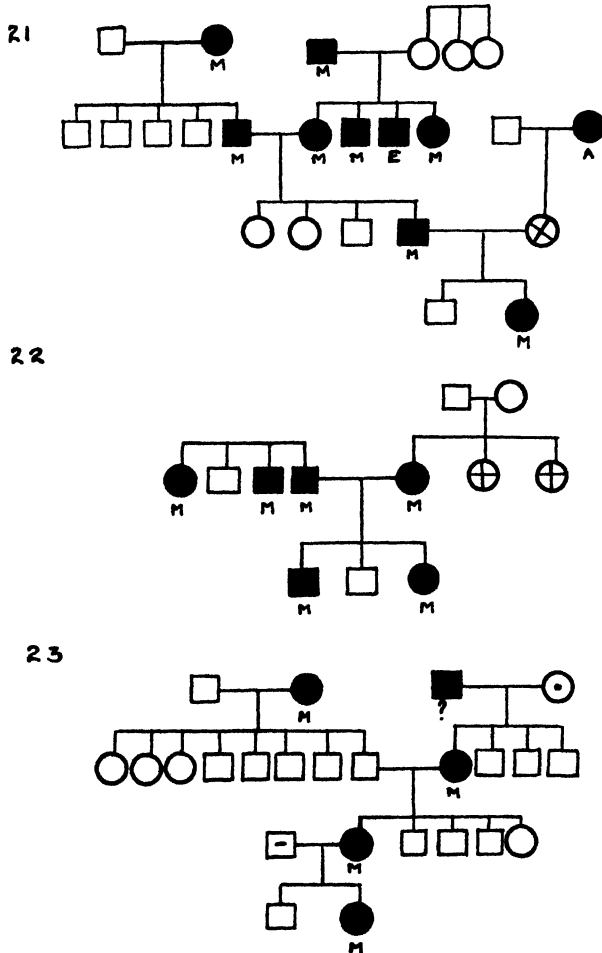
Obviously a cross of this kind will show at once what types of allergy are dominant, for if any allergy appears again in the  $F_1$  from such a cross its dominance is certain.

Unfortunately, as we have stated before, marriages of this type are not numerous. We have among our fifty-three pedigrees twelve matings of this



type (Nos. 1, 5, 12, 18, 29, 32, 34, 37, 38, 41, 42, 43). In pedigree 1, hay fever and migraine act as dominants, in No. 12, hay fever; in No. 29, urticaria and migraine, in No. 34, eczema, and in No. 43, asthma acts in a similar manner. Thus each of these five types of allergy may show dominance.

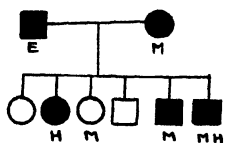
However, in spite of this dominance, not even half of the  $F_1$ , as we should expect if the allergic parent were heterozygous, develop the sensitivity. In family 29 a man affected by migraine and urticaria married into a negative family and had four children; one of the four had hay fever, urticaria and migraine, but the other three were negative. In family 32, a woman with migraine and hay fever married into a negative line and



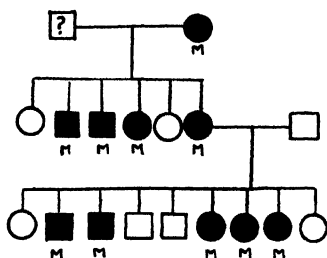
had two children, one of whom had eczema, the other hay fever. In family 18, a woman with asthma married into a negative line and had a daughter with migraine. In family 38 a man with hay fever and asthma married into a negative line and had nine children, of whom only one daughter had any trouble—hay fever and eczema. In family 34 a woman with eczema married into a negative line and had four children only one of whom showed any allergic trait, and this child had migraine, urticaria, and ec-

zema. In family 41 a man with migraine married into a negative line and had six children, four of whom were allergic, a son and a daughter each having hay fever, a son migraine, and a daughter urticaria and eczema.

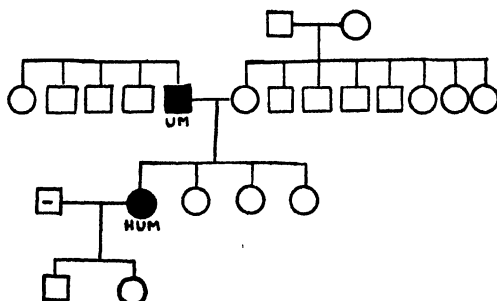
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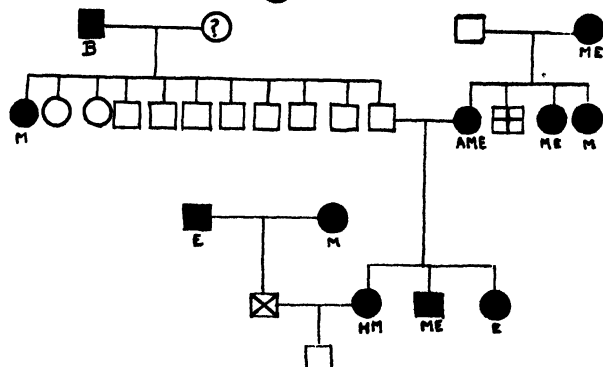
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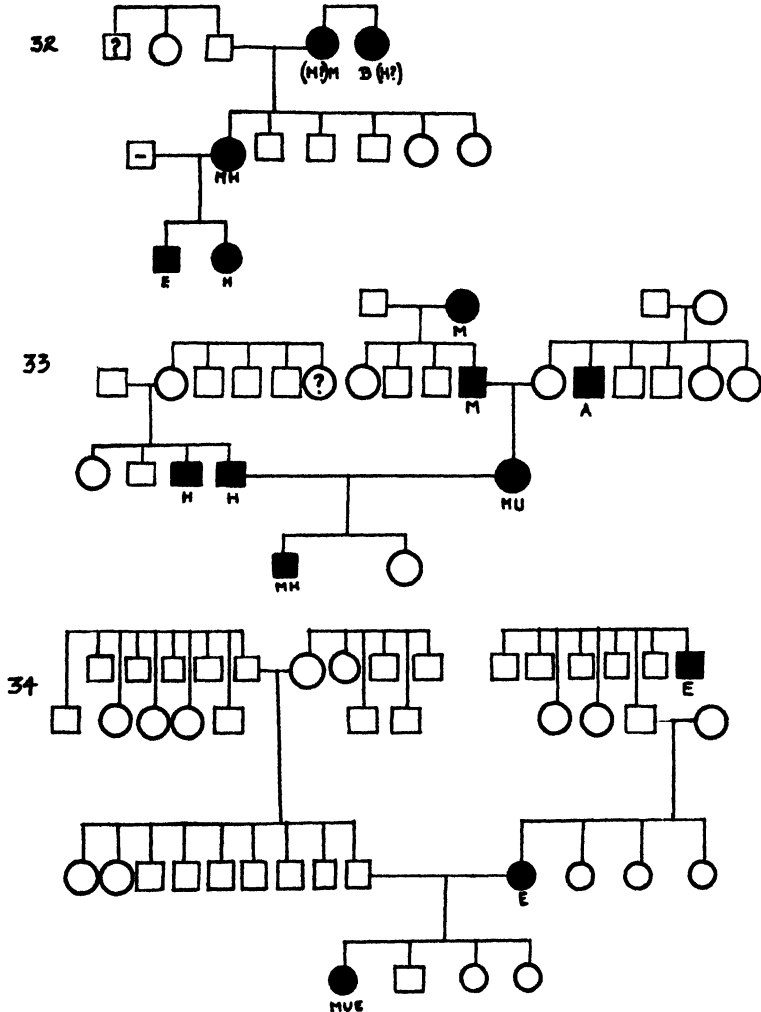


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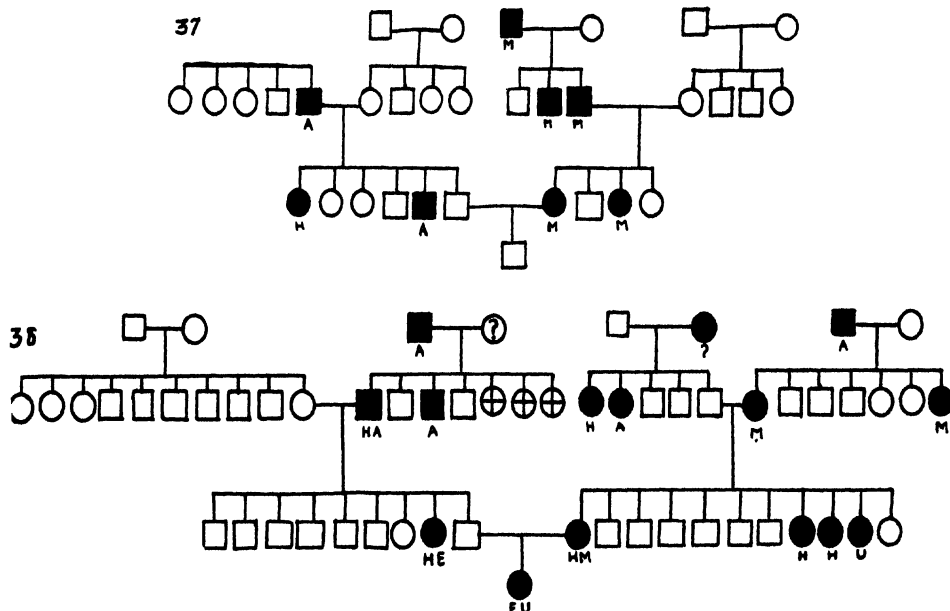
If we consider sensitivity as due to one gene and each allergy as due to a modifying gene, then we must assume that in each of these cases the negative line brought in the special modifiers necessary to produce the different types of allergy. If these modifiers exist, then, as has just been

shown, they must act as dominants when present in connection with the gene for sensitivity. Since these possible modifiers are dominant, the negative lines referred to above would have to bring in one or more dominant modifying genes for the different forms of allergy, these modifying genes being unable to express themselves without the gene for sensitivity which is lacking in the negative line. Following out this line of reasoning and assum-



ing that there is a specific modifying gene for each allergy, we would expect these dominant modifiers to cause new allergies in a fair percentage of the children. If the negative parent were heterozygous for the modifying gene, only half the children would have the new allergy. In any event we should never expect complete dominance, since we have shown that the dominance is variable. However, the new combinations in the  $F_1$  are of various

kinds, one individual having one new allergy, another a second, and still others having different combinations of them. Sometimes only a small percentage of the progeny show the dominance of a particular type of allergy, and in other cases the same allergy that has been shown in similar crosses to act as a dominant does not appear in the  $F_1$  although it may be present in the parent.



Another fact revealed by this type of mating is that a person with one form of allergy may transmit other forms to his or her children. Examples to illustrate this condition are to be found in many pedigrees and are of two kinds: Either the allergy not present in the parent may have been

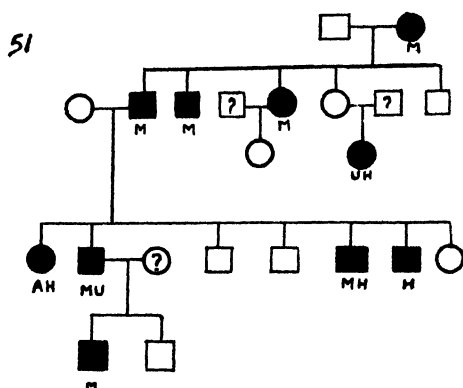
FAMILY NUMBER	NEGATIVE LINE CROSSED WITH	ALLERGY IN PARENT FAMILY BUT NOT IN PARENT	ALLERGIES IN PARENT AND ALSO IN $F_1$	ALLERGIES OF PARENT FAMILY ALSO IN $F_1$	NEW ALLERGY IN $F_1$
1	MH	A	MH	A	E
5	EM		M		
12	H		H		M
18	A	M		M	
29	UM		UM		H
32	MH		H		E
34	E		E		MU
38	HA		H		E
41	M	H	M	H	UE
42	HA		HA		BE
43	A		A		H





to consider that the negative line has brought in modifying factors for a new set of allergies. If such were the case these new allergies, being dominant as we have shown, should appear in a fairly large proportion of the  $F_1$ , a result not found to be true.

Matings between allergic families also establish this point. In family 8, migraine had appeared for two generations on both sides and no other allergy had been manifest. The father and mother, both free from allergy, had seven children, two of whom suffered from migraine, and another from five allergies, namely, migraine, eczema, asthma, urticaria, and hay fever. How was it possible for four allergies, apparently new to both sides of the family (three of which have been shown in our other crosses with negative lines to be dominants) to be present for two generations without manifesting themselves?



Other matings of two individuals, both from allergic families, also produce children with allergies which have not appeared in either family. Thus in families 2, 20, and 13 urticaria appears as a new allergy; in family 17 eczema; in family 31 hay fever. In this last case, as in the cases with negative lines, two allergies present in the family did not appear in any of the progeny. One parent had asthma and a grandparent had bronchitis, neither of which reappeared in the children.

These facts all lend support to the theory that there is only one gene for sensitivity and that this gene expresses itself in various ways in different individuals. This point, we believe, is definitely established by our crosses with negative lines. We must admit, however, that we have no explanation to offer for the five pedigrees in which there is only one allergy. It is of course possible that some of the individuals in these pedigrees may develop other allergies later in life.

We have one case of identical twins in family 14. Both of the twins have the same three forms of allergy. It is probable that the environment of the twins is very similar which may account for the occurrence of the

same allergies. Obviously, however, no conclusion can be drawn from one case and we shall hope to find other allergic identical twins.

In reviewing our charts, it is at once evident that migraine is more prevalent in females than in males. Among our pedigrees we find 64 males with migraine and 126 females so affected. At first sight, it might seem that migraine has something to do with the sex chromosome, for if it were dominant, as we have shown, but also sex linked, we should expect more migraine females than males. Our records show, however, that a son may inherit migraine from either his father or his mother. He may have migraine if his mother is from a negative family (family 41) and his father has migraine; or if his father is from a negative family and his mother has migraine (family 5). Apparently a son may be allergic if his parents are from allergic lines, regardless of which parent has migraine, or even if neither has that special form of allergy. The following chart makes this matter clear. In these cases both father and mother are most probably from allergic families. (Note: See p. 134, for meaning of letters. [. .] no allergy.)

SON	FATHER	MOTHER	FAMILY
M	..	M	15, 17, 21, 51
M	..	MEA	31
M	M	..	51, 21
M	H	MU	33
M	E	M	26
M	..	HB	3
M	..	..	8, 17
M		A	15
M	M	M	21, 22

It has been suggested that allergy might be due to a factor which would bring it about in females when in the heterozygous condition, but that the homozygous presence of the gene would be necessary to cause the allergy to appear in the male. A condition just opposite to this exists in cattle where if the gene for "hornless" is present in the duplex condition in a female she is hornless, whereas only the simplex condition is necessary to make a hornless male. The inheritance of migraine obviously is not comparable to this case, for a male may develop migraine when he inherits the gene for sensitivity from only one side, that is, when he is heterozygous. Since our records show that with the same inheritance females are more likely to have this allergy than males, the reason for this difference must probably be sought in the physiological differences between the sexes. One might conjecture that the hormones related to sex may be the cause of the different proportions.

The conclusions of this paper are in harmony with the work of most other students of the inheritance of allergic diseases. Except for the works

of ADKINSON and BUCHANAN the types of allergy have been considered as due to dominant factors. ADKINSON's results can be explained as due to a dominant gene, dependent for its expression to some extent upon the environment. Migraine has been regarded as a sex-linked character by several writers because of the large number of females who have it. However, we have shown that migraine may be inherited by the son from the father when the mother's line is negative and therefore cannot be sex-linked. Also migraine is interchangeable with the other allergies and acts as the expression of the same gene for sensitivity which obviously has no connection with sex. Since allergic women are apt to suffer from these headaches during the menstrual period (a fact which has been referred to a number of times in the literature and which we also have observed), the preponderance of migraine females can best be explained as correlated in some way with the sex hormones and with physiological processes. Finally, we have found no connection (as did STUDY and GÄNSSLEN) between gout and the allergic diseases mentioned.

We have shown that any allergy may act as a simple dominant, and an allergic individual may transmit either the same or another type of allergy. There is thus no regularity in the transmission of the different types of allergy and the individual known to carry the gene for sensitivity may be normal. Our evidence thus shows that most probably allergy is not due to multiple factors but rather to the expression of a single gene for sensitivity which manifests itself in different ways, depending to some extent upon the environment of the individual. The gene for allergy is not sex linked. The greater number of migraine females cannot be explained as due to a sex-linked gene.

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# INHERITANCE IN BARLEY<sup>1</sup>

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## INTRODUCTION

In recent years, considerable information on inheritance in barley has been obtained. At present, some five or six different linkage groups have been found. Various factors have been located in the different groups but few have been mapped. The present studies involve several factor pairs which previously have not been located in any of the groups studied.

## REVIEW OF LITERATURE

The review of literature on the genetics of barley is confined to the characters studied in this paper.

### *Chlorophyll deficiencies*

Colsess V described by ROBERTSON and DEMING (1930) is a chlorophyll deficient plant which grows to maturity but is less vigorous than the normal green type. The plant color according to RIDGEWAY, plate XVII, is dull green yellow. The chlorina seedlings are recessive to normal green.

The interrelationship of the factor pair (*F<sub>c</sub>f<sub>c</sub>*) for green *versus* chlorina seedlings in Colsess V has been tested (ROBERTSON and DEMING 1930) with the following factor pairs: (*Kk*) for hoods *versus* awns in linkage group IV, (*A<sub>1</sub>a<sub>1</sub>*) for green *versus* white seedlings in Trebi found in linkage group II, (*Ff*) for green *versus* chlorina seedlings in Minnesota 84-7 located in linkage group I (ROBERTSON, DEMING and KOONCE 1932).

### *Inheritance of other characters*

A rather intensive review of the inheritance of the following factor pairs is given by DAANE (1931). Hulled *versus* naked, long *versus* short haired rachilla, hoods *versus* awns, and six-row *versus* non-six-row all segregated in simple Mendelian ratios. He also summarizes the published data on six-row *versus* non-six-row. The *Vv* factor differentiates between six-row and non-six-row but the intermedium factor pair *Ii* influences the non-six-row head type in *F*<sub>2</sub> and *F*<sub>3</sub>. He concludes that "Defeciens," "distichon," and

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"vulgate" form an allelomorphic series but that intermedium or low fertility reported by HARLAN and HAYES, GRIFFEE, and ROBERTSON is not an allelomorph of the above mentioned series.

# MATERIAL AND METHODS

This paper presents studies of the inheritance of simple Mendelian factor pairs and their possible linkage relations. The following symbols were used to designate the different factor pairs studied.

Non-six-row <i>versus</i> six-row	<i>Vv</i>
Black <i>versus</i> white glume color	<i>Bb</i>
Hulled <i>versus</i> naked caryopsis	<i>Nn</i>
Hoods <i>versus</i> awns	<i>Kk</i>
Intermedium <i>versus</i> non-intermedium	<i>Ii</i>
Long <i>versus</i> short haired rachilla	<i>Ss</i>
Green <i>versus</i> white seedlings	<i>Aa</i>
Green <i>versus</i> yellow seedlings	<i>Xx</i>
Green <i>versus</i> Chlorina seedlings	<i>Ff</i>
Purple <i>versus</i> white straw color	<i>P<sub>r</sub> p<sub>r</sub></i>

The parental varieties were differentiated by the following factor pairs:

TABLE 1  
*List of factor pairs found in the different parents.*

NEPAL	CHEVALIER	BLACK HULLESS	TREBI	COLSESS V	NILSSON-EHLE NO. 2
<i>vv</i>	<i>VV</i>	<i>vv</i>	<i>vv</i>	<i>vv</i>	<i>VV</i>
<i>bb</i>	<i>bb</i>	<i>BB</i>	<i>p<sub>r</sub>p<sub>r</sub></i>	<i>p<sub>r</sub>p<sub>r</sub></i>	<i>P<sub>r</sub>P<sub>r</sub></i>
<i>nn</i>	<i>NN</i>	<i>nn</i>	<i>bb</i>	<i>bb</i>	<i>bb</i>
<i>KK</i>	<i>kk</i>	<i>KK</i>	<i>NN</i>	<i>NN</i>	<i>NN</i>
<i>ss</i>	<i>ss</i>	<i>kk</i>	<i>kk</i>	<i>KK</i>	<i>kk</i>
<i>X<sub>n</sub>x<sub>n</sub></i>	<i>ii</i>	<i>II</i>	<i>II</i>	<i>II</i>	<i>..</i>
	<i>SS</i>	<i>SS</i>	<i>ss</i>	<i>ss</i>	<i>SS</i>
		<i>A<sub>1</sub>a<sub>1</sub></i>	<i>fcfc</i>	<i>fcfc</i>	<i>A<sub>1</sub>a<sub>1</sub></i>

## Methods of conducting experiments

Methods similar to those described in a previous publication (ROBERTSON 1929) were used. The same symbols were used to interpret characters as those used in previous publications (ROBERTSON 1929, ROBERTSON and DEMING 1930, ROBERTSON, DEMING and KOONCE 1932, DAANE 1931). Additions to the latter list were made for the following character pairs: Intermedium *versus* non-intermedium (*Ii*) and Purple *versus* white straw color (*P<sub>r</sub> p<sub>r</sub>*).

## INHERITANCE OF SIMPLE MENDELIAN FACTOR PAIRS

In the cross between Colsess V and Chevalier, the parents apparently differed by two pairs of factors for fertility of the lateral florets and the

results were entirely similar to those obtained by HARLAN and HAYES in 1920. The genotypes of the  $F_2$  plants were determined by breeding behavior in  $F_3$ , the particular purpose being to find which of the  $F_2$  plants carried the intermedium factor. Table 2 gives the type of segregations which were obtained. The  $F_2$  genotypes belonging to classes 4 and 7 evidently carried the intermedium factor in a recessive condition. The remaining classes had the dominant condition for the intermedium factor. Class 1 which bred true for six-row could not be used in this material, as it could not be differentiated for the presence or absence of the intermedium factor. Using the material in classes 2 to 7, a ratio of 1184  $F_2$  plants carrying dominant  $I$  and 447 of the recessive type were obtained. These classes were used later in the study of the interaction between the chlorina factor pair and the intermedium factor pair.

TABLE 2  
*F<sub>2</sub> segregating classes and F<sub>2</sub> genotypes of non-six-row plants from a cross Colseess V (vulgare) × Chevalier (distichon).*

CLASS	F <sub>2</sub> SEGREGATING CLASSES	F <sub>2</sub> GENOTYPE
2	6-row and intermedium	VvII
3	6-row intermediate, intermedium and 2-row	VvIi
4	6-row and 2-row	Vvii
5	Homozygous for intermedium	V'V'II
6	Intermedium and 2-row	V'V'iI
7	2-row	V'V'ii

The inheritance of the character pair green *versus* white seedlings was studied in a cross between Colseess × Black Hulless and Black Hulless × Trebi. A simple Mendelian segregation was obtained (table 3).

The inheritance of straw color was studied in a cross between Nilsson Ehle No. 2 and Trebi. The data in table 3 indicate that purple *versus* white straw color is the result of the interaction of a simple Mendelian factor pair.

TABLE 3  
*Inheritance of simple Mendelian characters.*

FACTOR PAIRS	CHARACTERS	A	a	DEV. IN NUMBERS	DEV. P.E.
<i>Ii</i>	Intermedium <i>versus</i> non-intermedium	1184	447	39.25	3.3
<i>A<sub>1</sub>a<sub>1</sub></i>	Green <i>versus</i> white seedlings	1350	428	16.50	1.3
<i>P<sub>1</sub>p<sub>1</sub></i>	Purple <i>versus</i> white straw	676	217	6.25	0.7

#### INTERRELATION OF SIMPLE MENDELIAN FACTOR PAIRS

The factor pair ( $F.f.$ ) and its relationship with the following linkage groups III ( $Nn$ ), IV ( $Kk$ ) and V ( $Ss$ ) (DAANE 1931), (ROBERTSON 1932) was studied.

*Green versus chlorina seedling ( $F_c f_c$ ) and covered versus naked caryopsis ( $Nn$ )*

The interrelationship of the factor pair ( $F_c f_c$ ) and ( $Nn$ ) was studied in a cross between Colsess V and Nepal. The number of chlorina plants was lower than expected on the basis of a segregation for 3 green to 1 chlorina. The  $\chi^2$  test for independence was used. The data presented in table 4 indicate that the factor pairs ( $F_c f_c$ ) and ( $Nn$ ) are inherited independently of each other.

*Green versus chlorina seedlings ( $F_c f_c$ ) and the intermedium versus non-intermedium ( $Ii$ )*

The intermedium and non-intermedium classes in table 3 were used for the study. They were regrouped for plant color, and a close fit to a 9:3:3:1 ratio was obtained (table 4). The data presented indicate that the factor pairs ( $F_c f_c$ ) and ( $Ii$ ) are inherited independently of each other.

*Green versus chlorina ( $F_c f_c$ ) and long versus short haired rachilla ( $Ss$ )*

The interrelationship of the factor pairs ( $F_c f_c$ ) for green *versus* chlorina seedlings and the factor pair ( $Ss$ ) which is located in group V was studied in a cross between Colsess V and Nepal 1. The observed and calculated ratios for independence are given in table 4. The  $\chi^2$  test indicates that the factor pairs ( $F_c f_c$ ) and ( $Ss$ ) are inherited independently of each other.

The data already presented show that the factor pair ( $F_c f_c$ ) for green *versus* chlorina seedlings in Colsess V is inherited independently of factors found in the following groups: Group I (1932); Group II (1930); Group III (Present paper); Group IV. (1930) and Group V (1932). The factor pair ( $F_c f_c$ ) is, however, linked with the factor pair ( $Y_c y_c$ ) for green *versus* virescent seedlings in Coast III (1932).

*Intermedium versus non-intermedium ( $Ii$ ) and hoods versus awns ( $Kk$ )*

The interrelationship of the factor pairs ( $Ii$ ) for intermedium *versus* non-intermedium and ( $Kk$ ) for hoods *versus* awns was studied in a cross between Colsess V and Chevalier. The factors went into the cross in the following order:  $KKII$  in Colsess and  $kkii$  in Chevalier. The  $F_2$  segregation should, therefore, be in the coupling phase.

In testing the interrelationship of the factor pairs ( $Kk$ ) and ( $Ii$ ) the following segregations were obtained (table 5).

If linkage occurred it would be in the coupling phase, since the factor pairs went into the cross as  $KKII$  and  $kkii$ . The above data indicate such a linkage. IMMER's tables for calculating linkage intensities were used and a crossover percentage of  $15.12 \pm 0.65$  was obtained; when the observed



TABLE 4  
*Character pairs independently inherited.*

CROSS	CHARACTERS	PHASE	AB*	Ab	aB	ab	$\chi^2$	P
Colseass V×Nepal 1	$F_c f_c$ $Nn$	Repulsion Observed	1432.0	446.0	379.0	127.0	0.40	0.53
		Calculated	1426.6	451.4	384.4	121.6		
Colseass V×Chevalier	$F_c f_c$ $Ii$	Coupling Observed	887.0	339.0	299.0	105.0	4.86	0.18
		Calculated	916.9	305.6	305.6	101.9		
Colseass V×Nepal 1	$F_c f_c$ $Ss$	Coupling Observed	1392.0	486.0	387.0	119.0	1.17	0.29
		Calculated	1401.4	476.6	377.6	128.4		
Colseass×Chevalier	$Vv$ $Kk$	Repulsion Observed	1302.0	464.0	405.0	137.0	0.20	0.65
		Calculated	1306.0	460.0	401.0	141.0		

$\chi^2$  Calculated for independence.

\* The classes for the character pairs are headed by the general symbols AB, Ab, aB and ab.

TABLE 5

*F<sub>2</sub> segregations of a cross between Colsess V and Chevalier. The F<sub>2</sub> classes for the (Ii) factor pair were determined from F<sub>2</sub> segregations.*

	HOODED INTER- MEDIUM	HOODED 2-ROW	AWNED INTER- MEDIUM	AWNED 2-ROW	$\chi^2$	P
Observed	1078.0	129.0	106.0	318.0		
Calculated 9:3:3:1	917.4	305.8	305.8	101.9	718.80	very small
Calculated 15.12 percent crossover	1109.3	114.0	114.0	293.8	5.42	.07

ratio is compared with a calculated ratio with 15.12 percent crossing over, a close fit was obtained.

Since there is a linkage of the factor pairs (*Kk*) and (*Ii*) and it is in the coupling phase, this should prove the genetic constitution of the parents for the (*Kk*) and (*Ii*) factors. Table 5 shows a coupling type of linkage and, therefore, (*KK*) and (*II*) must have gone into the cross in Colsess since it is hooded, and *kkii* must have gone into the cross in Chevalier since it is awned and non-intermedium. In a previous publication, ROBERTSON (1929) obtained a ratio of 1 six-row, 2 intermediate and 1 two-row in a cross between Colsess I and *H. distichon nigrinudum*. The two-rowed parent evidently has the genetic constitution (*VVII*) and Colsess I has the genetic constitution (*vvII*). In this case the only factor pair segregating would be (*Vv*) and a simple Mendelian segregation would be obtained in *F<sub>2</sub>*, proving the constitution of *H. distichon nigrinudum* to be (*VVII*), since Colsess has already been shown to have the (*II*) factor in the dominant condition. As has already been stated of the cross Colsess V and Chevalier, classified as *H. distichon* by HARLAN (1918), seven classes were obtained in *F<sub>2</sub>* showing that two factor pairs are involved: the (*Vv*) factor pair for non-six-row *versus* six-row and the (*Ii*) factor pair for intermedium *versus* non-intermedium. Since the Colsess parent has the genetic constitution (*vvII*) the genetic constitution of Chevalier would be (*VVii*) giving a *F<sub>1</sub>* plant which would be heterozygous for both factor pairs. Since two types of segregation were obtained from crosses of Colsess  $\times$  *distichon* the indications are that the (*Ii*) factor pair is independent of the allelomorphic series for six-row, "*distichon*" and "*deficiens*."

From the same crosses where (*Ii*) and (*Kk*) indicated a linkage the inter-relationship of the factor pairs (*Kk*) and (*Vv*) were studied also. For this purpose, the *F<sub>2</sub>* data for rows were again used. The groups were classed as follows: six-rowed hooded; six-rowed awned; non-six-rowed hooded and non-six-rowed awned. Table 4 gives the different segregating groups for the factor pairs (*Vv*) and (*Kk*). From the data obtained it may be concluded that the factor pairs (*Vv*) and (*Kk*) are inherited independently of each other.

Since there is no indication of linkage between the factor pairs ( $Kk$ ) and ( $Vv$ ) it is also evident that the factor pairs ( $Vv$ ) and ( $Ii$ ) are in different linkage groups. This is further borne out by earlier studies which show ( $Vv$ ) and ( $Kk$ ) to be located in different groups (ROBERTSON 1929, DAANE 1931).

*Interrelation of green versus white seedlings ( $A_{ba_b}$ )  
and long versus short haired rachilla ( $Ss$ )*

The interrelationship of the factor pair ( $A_{ba_b}$ ) with ( $Kk$ ) ( $Nn$ ) and ( $Ss$ ) was studied in crosses between Colsess  $\times$  Black Hulless and Black Hulless  $\times$  Trebi. In one of the former crosses only the Black Hulless parent was heterozygous for white seedlings and the relationships of the factor pair ( $A_{ba_b}$ ) were studied in this cross. The following simple Mendelian segregations were obtained:

TABLE 6  
*Inheritance of simple Mendelian characters.  $F_2$  plants heterozygous for factor pair ( $A_{ba_b}$ ).*

CHARACTER		A	a	DEVIATION IN NUMBERS	DEVIATION P.E.
$Kk$	Hoods <i>versus</i> Awns	335	115	2.5	0.40
$Nn$	Covered <i>versus</i> naked	331	119	6.5	1.05
$Ss$	Long <i>versus</i> short haired rachilla	300	150	37.5	6.05
* $Ss$	Long <i>versus</i> short haired rachilla	683	216	8.75	1.00

\* The families giving this segregation were homozygous for green.

In table 6 it will be seen that long *versus* short haired rachilla does not fit the calculated 3 to 1 ratio very well. The families from which the first segregation is obtained were also heterozygous for seedling color. The second count which fits a calculated 3:1 ratio was obtained from two pure green families. This would indicate that the character long *versus* short haired rachilla is due to the interaction of a simple Mendelian factor pair, but the segregation is influenced by the presence of the factor pair ( $A_{ba_b}$ ) for green *versus* white seedlings.

The other two factor pairs ( $Kk$ ) and ( $Nn$ ) seem to fit the calculated 3:1 ratio very well. Since the factor pair ( $A_{ba_b}$ ) was also present in the families in which the above characters were studied and has no influence on the segregation one can conclude that the factor pairs ( $Kk$ ) and ( $Nn$ ) are inherited independently of the factor pair ( $A_{ba_b}$ ).

A further study of the interrelationship of the factor pairs ( $A_{ba_b}$ ) and ( $Ss$ ) was made in a cross between Black Hulless and Trebi.

The  $F_2$  data when studied in relationship to the two factor pairs show a poor fit to a calculated 9:3:4 ratio which is similar to the data found in the Colsess cross.

TABLE 7  
*F<sub>2</sub> segregation of long versus short haired rachilla and green versus white seedlings.*

CROSS		GREEN LONG	GREEN SHORT	WHITE	$\chi^2$	P
Colsess×Black Hulless 9:3:4	Observed	300	150	149	16.7	0.0003
	Calculated	336.9	112.3	149.8		
Trebi×Black Hulless 9:3:4	Observed	973	483	517	52.58	very small
	Calculated	1109.8	369.9	493.3	..	..

Since long haired rachilla went into the cross in the heterozygous parent a decrease in the number of long haired rachilla plants would be expected if the factors for white seedlings and long haired rachillas were linked. COLLINS' formula for use when one of the classes is missing was used to determine the crossover percentage.

$$p = \sqrt{\frac{AB - 2Ab}{AB - Ab}}$$

In the Colsess×Black Hulless cross no crossing over was obtained. In the Trebi×Black Hulless cross a value of 6.93 percent was obtained. The fit of the observed to calculated ratio is given for the Trebi×Black Hulless in table 8.

TABLE 8  
*Observed and calculated ratios for 0 and 6.93 percent crossing over as determined from F<sub>2</sub> data.*

CROSS	GREEN LONG	GREEN SHORT	WHITE	$\chi^2$	P
Colsess×Black Hulless Observed	300	150	149	..	..
Calculated 0 percent crossover	299.50	149.75	149.75	.005	very large
Trebi×Black Hulless Observed	973	483	517	..	..
Calculated 6.9 percent crossover	988.9	490.9	493.2	1.53	0.48

Since F<sub>2</sub> data where only three classes are available give results which are considerably less reliable than F<sub>2</sub> data with four classes distinguishable, the progeny of the green plants with long haired rachillas were grown in F<sub>3</sub>, and the F<sub>2</sub> genotypes determined for the factor pairs (Ss), long *versus* short haired rachillas and (A<sub>b</sub>a<sub>b</sub>) green versus white seedlings. Table 9 gives the results obtained from the F<sub>3</sub> data.

TABLE 9  
*Proportion or number of F<sub>2</sub> genotypes as determined from F<sub>3</sub> breeding behavior.*

CROSS	AASS	AASs	AaSS	AaSs	$\chi^2$	P
Colsess×Black Hulless Observed	3	53	53	180	..	..
Calculated 1:2:2:4 ratio	32.1	64.2	64.2	128.4	51.02	very small
Trebi×Black Hulless Observed	56	319	260	1017	..	..
Calculated 1:2:2:4 ratio	183.6	367.1	367.1	734.2	235.1	very small

The F<sub>2</sub> data further indicate that the factor pairs (Ss) and (A<sub>b</sub>a<sub>b</sub>) are linked.

When the  $F_2$  genotype classes are tested for linkage a crossover value of 23 percent for the Colsess cross and 26 percent for the Trebi cross is obtained.

TABLE 10  
*F<sub>2</sub> genotype classes and calculated ratios with 23 and 26 percent crossing over.*

CROSS	AASS	AASs	AaSS	AaSs	$\chi^2$	P
Colsess $\times$ Black Hulless	3	53	53	180	..	..
Calculated 23 percent crossover	7.45	49.86	49.86	181.8	3.07	0.38
Trebi $\times$ Black Hulless	56	319	260	1017	..	..
Calculated 26 percent crossover	54	307.4	307.4	983.2	8.98	0.02

The P value for the Trebi  $\times$  Black Hulless cross is small and the high  $\chi^2$  value is caused by the *AaSS* group. This discrepancy may have been due to slight errors in classification and some of the plants from the *AaSS* class may have been placed in the *AaSs* class. However, the observed fits the calculated ratio with 23 percent to 26 percent crossing over much better than a ratio calculated for independence of the factor pairs (*A<sub>b</sub>a<sub>b</sub>*) and (*Ss*).

When we test the fit of the  $F_2$  data to calculated ratios with 23 percent and 26 percent crossing over, a good fit is obtained also.

TABLE 11  
*F<sub>2</sub> segregation for long versus short haired rachillas and green versus white seedlings.*

CROSS	GREEN LONG	GREEN SHORT	WHITE	$\chi^2$	P
Colsess $\times$ Black Hulless Observed	300.00	150.00	149.00	.	.
Calculated Ratio 22.75 percent crossover	307.25	142.00	149.75	0.63	large
Trebi $\times$ Black Hulless	973.00	482.00	517.00	..	..
Calculated Ratio 26 percent crossover	1019.80	459.90	493.30	4.44	0.11

It is apparent that the factor pairs (*A<sub>b</sub>a<sub>b</sub>*) and (*Ss*) are linked with about 26 percent crossing over.

*Purple versus colorless straw (P, p,) and non-six-row  
versus six-row (Vv)*

The interrelationship of the factor pair for six-rowed *versus* non-six-rowed and straw color was studied in a cross between the selection Nilsson-Ehle No. 2 and Trebi. There was some indication of linkage. Colored straw and two-rows went into the cross in selection No. 2 and colorless straw and six-row went into the cross in Trebi.

In studying the interrelationship of straw color and rows,  $F_2$  data were used to determine the  $F_2$  genotypes. The results of this study are given in table 12.

The results indicate that the factor pair (*Vv*) for non-six-row *versus* six-row is linked with factor pair (*P,p*) for purple *versus* colorless straw with about 9.0 percent crossing over.

TABLE 12

*F<sub>2</sub> genotypes on the basis of F<sub>2</sub> breeding behavior in a cross segregating for six-rowed versus non-six-rowed and purple straw versus colorless straw compared with a calculated 9:3:3:1 ratio and a ratio calculated with 9 percent crossing over.*

	NON-SIX-ROW		SIX-ROW		$\chi^2$	P
	PURPLE	WHITE	PURPLE	WHITE		
Observed	636.00	45.00	40.00	171.00	..	..
Calculated 9:3:3:1	501.75	167.25	167.25	55.75	460.00	very small
Calculated 9 percent crossing over	630.66	38.34	38.34	184.66	2.28	0.52

In order to check the possibility of linkage with the intermedium factor pair, the following  $F_2$  classes were grouped: 2 = ( $II$ ), 3 = ( $Ii$ ), 5 = ( $II$ ), 6 = ( $Ii$ ) as intermedium and the other classes, four and seven, were grouped as 2-row. The six-row class had to be discarded as no distinction of the ( $II$ ), ( $Ii$ ) and ( $ii$ ) combinations could be made. The results obtained due to the discarding of the six-row colorless class reduced the number of individuals in the colorless class and gave a ratio which was not a normal 9:3:3:1 ratio but fitted two 3:1 ratios very well.

TABLE 13

*F<sub>2</sub> segregation of purple versus white and intermedium versus two-row.*

	PURPLE		WHITE		$\chi^2$	P
	INTERMEDIUM	2-ROW	INTERMEDIUM	2-ROW		
Observed ratio	458.0	178.0	34.0	11.0	.	.
Calculated ratio for independence	459.5	176.5	32.5	12.5	0.2668	0.60

The above data further indicate that the factor pairs ( $Vv$ ) and ( $Ii$ ) are inherited independently of each other.

#### SUMMARY

1. The inheritance of the following character pairs was explained on a simple Mendelian basis. Intermedium *versus* non-intermedium ( $Ii$ ), green *versus* white seedlings in Black Hulless ( $A_a$ ,  $a_a$ ) and purple *versus* white straw color ( $P$ ,  $p$ ).

2. The interrelationship of the factor pairs ( $Ff_c$ ) for green *versus* chlorina seedlings and factor pairs in linkage groups III, IV and V were studied. (a) The factor pair ( $Ff_c$ ) was found to be inherited independently of the following factor pairs: ( $Nn$ ) Group III, ( $Kk$ ) Group IV and ( $Ss$ ) Group V. It has previously been found to be inherited independently of factors in Group I, II and VI.

3. The factor pair ( $Ii$ ) was found to be linked with the factor pair ( $Kk$ ) with  $15.12 \pm .065$  percent crossing over.

4. The factor pair ( $Vv$ ) was found to be inherited independently of the factor pair ( $Kk$ ), indicating that the factor pairs ( $Vv$ ) and ( $Ii$ ) are not in the same linkage group.

5. The white seedling factor pair ( $A_1a_1$ ) was found to be inherited independently of the factor pairs ( $Kk$ ) and ( $Nn$ ) but was found to be linked with the factor pair ( $Ss$ ) with a percentage crossover of about 26.

6. The factor pair ( $P_r p_r$ ) was found to be linked with the factor pair ( $Vv$ ) with a crossover value of  $9.0 \pm 0.68$ . The straw color factor pair was also found to be inherited independently of the factor pair ( $Ii$ ).

7. The present study adds another linkage group to the six already found. Chlorina ( $F_c f_c$ ) was found to be inherited independently of at least one factor pair in each of the already known groups.

The factor pair ( $Ii$ ) has been added to Group IV.

The factor pair ( $A_1a_1$ ) has been added to Group V and the factor pair ( $P_r p_r$ ) has been added to Group I.

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INHERITANCE OF COLOR PATTERNS IN THE GROUSE  
LOCUST *ACRYDIUM ARENOSUM*  
BURMEISTER (TETTIGIDAE)<sup>1\*</sup>

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INTRODUCTION

Studies of inheritance in the grouse locusts (Tettigidae) have hitherto been confined mainly to the more rapidly breeding southern (United States of America) species, *Paratettix texanus* Hancock, *Apotettix eurycephalus* Hancock, *Telmatettix aztecus* Saussure, and *Tettigidea parvipennis pennata* Morse (NABOURS 1929, Bibliography). Four succeeding generations of any of these may be bred in the greenhouse within a year, as against one, or at best, only two generations annually of the more northern varieties and species of this subfamily.

The experiments herein reported were begun in February, 1925, when E. H. INGERSOLL, on an unseasonably warm day, collected some specimens of *Acrydium arenosum* in a wooded area, at the junction of the Kansas and Blue rivers, about two miles southeast of Manhattan, Kansas. Practically all of the specimens used have come from this region. The project was carried on one year by NABOURS, then for one year in cooperation with NELLE HARTWIG (NABOURS and HARTWIG abstract 1926; Master's thesis by HARTWIG 1927, unpublished); then for four years (1927-1931) mainly by IVA LARSON (NABOURS and LARSON abstract 1929; and Master's thesis by LARSON 1929, unpublished). We are much indebted to MARJORIE DEAN for assistance in recording, and to S. FRED PRINCE and RUTH CLENCY for the drawings in the plate.

BIOLOGY OF *Acrydium arenosum*

The species *Acrydium arenosum*, according to HANCOCK (1902), is distributed over the southern states (United States of America), and as far north as in Nebraska and Iowa. However, during eighteen collecting trips into southern Texas and Louisiana, extending over the past twenty-three years, none of this species has been found in such areas as contained *Paratettix texanus* in large, and *Apotettix eurycephalus* in smaller, numbers. *A. arenosum* are found in considerable numbers in the region of Manhattan, Kansas, mainly in wooded areas, along the streams.

<sup>1</sup> Contribution 146 from the Department of Zoology, KANSAS STATE AGRICULTURAL EXPERIMENT STATION, Manhattan, Kansas.

\* The cost of the accompanying color plate is paid by the GALTON AND MENDEL MEMORIAL FUND.



In superficial appearance the members of this species differ only slightly from those of *P. texanus* and *A. eurycephalus*, the real distinctions consisting of smaller, though taxonomically important, features. They are, perhaps, even more varied in the elementary and hybrid dominant color patterns of the pronota and femora of the posterior legs than are the southern species named above (see plate 1). They also vary greatly with respect to sizes, and there is the usual dimorphism of lengths of the pronota and wings, with occasional intermediates. Nothing has been done toward determining the causes of the variations in sizes, and in wings and pronota lengths or to determine the inheritance of these characteristics. With respect to these features they resemble *P. texanus* (NABOURS 1914), wherein the lengths of wings and pronota were found to be probably correlated with the length of the growth periods; they were preponderantly long in the spring and early summer when growth was more rapid, and short in the late summer and fall when the growth rate was slower.

The members of this species, as well as other varieties and species of grouse locusts, in the region of Manhattan, Kansas, and farther north, in contrast with southern ones, hibernate during the cold season, usually from some time in October till in April, with practically no activity except on unseasonably warm days. The duration of the period of hibernation appears to depend upon the latitude, or length of the season. They hibernate in tufts of grass, under stones, leaves, pieces of wood, etc. They have been observed to survive weather below 0° F. The mortality is usually high during the period of hibernation, due probably as much to desiccation as to low temperatures.

Even in the greenhouse, *A. arenosum*, except when continuously exposed to mercury vapor, or intense white light, do not breed at all during the period from September to April, inclusive. They are carried through the winter with great difficulty, and then the prolificacy of the few survivors seems to be much impaired. We have failed in varied and repeated efforts to produce, either indoors, outdoors, or in refrigerators, conditions tolerant for the kind of hibernation that would carry the pedigreed stock over the winter in good condition for further breeding the following spring. Therefore, mainly new and unknown stock, with females sometimes already mated to anonymous males, had to be brought in each year for the one, or two generations. Thus, although exhibiting great diversity of elementary and hybrid, dominant color patterns, and probably also many heritable form and size variations, this species affords genetic data slowly and very meagerly.

#### THE EFFECTS OF EXPOSURE TO CONTINUOUS LIGHT

Specimens of *A. arenosum* were kept under the continuous light of a mercury vapor lamp, at distances of 30 to 36 inches, with 12-mesh wire

intervening, for various lengths of time, during a period of about two years. While some of the results of this experiment were obscure, a few pairs of those exposed to the light produced offspring in the late autumn and early winter, as against no progeny from many controls living at approximately the same temperatures, under the ordinary conditions of the greenhouse. It was also ascertained that nymphs, while exposed to the mercury lamp, ate algae (their normal food) readily, were quite active, and grew rather rapidly to the imago, even in the late fall and winter, as compared with the controls which did not grow sufficiently to induce any ecdyses till late in April, or in May. Subsequently, CURTIS SABROSKY, working in our laboratory, has ascertained that quite intense light from 200-watt, filamentous, clear bulbs produces practically the same effects as does the mercury vapor lamp, the temperatures under both kinds of lights and for the controls, with comparable variations, running nearly the same.

#### THE COLOR PATTERNS

Thus far twenty-four distinct, elementary, dominant color patterns of the pronota and the femora of the posterior legs, and the generalized, mottled gray recessive, or "wild type" (+/+), have been employed in the experiments. The system of naming the patterns is similar to that used for *P. texanus* and *A. eurycephalus* (NABOURS 1929, Bibliography). Several of these patterns resemble, to a greater or less degree, some of those of the species named. However, there are considerable differences in the linkage relations of such similar patterns among these species, respectively, as a comparison discloses (see table 1 and the plate 1).

The following is an attempt to describe the elementary color patterns, all dominant except the first:

1. +/+. Mottled, or smooth gray. Comparable with the same pattern in most grouse locusts so far studied. Recessive to all other patterns yet examined in this species.

2. *B*. Pronotum chalky white, becoming uneven gray posteriorly. Reddish brown tipped pronotum and wings. Femora of anterior legs white; those of hind legs unevenly reddish to orange brown.

3. *Bil*. Creamy white, or yellow, bilateral lines extending from anterior carinae to humeral angle, and thence posteriorly about two-thirds of distance to end of pronotum.

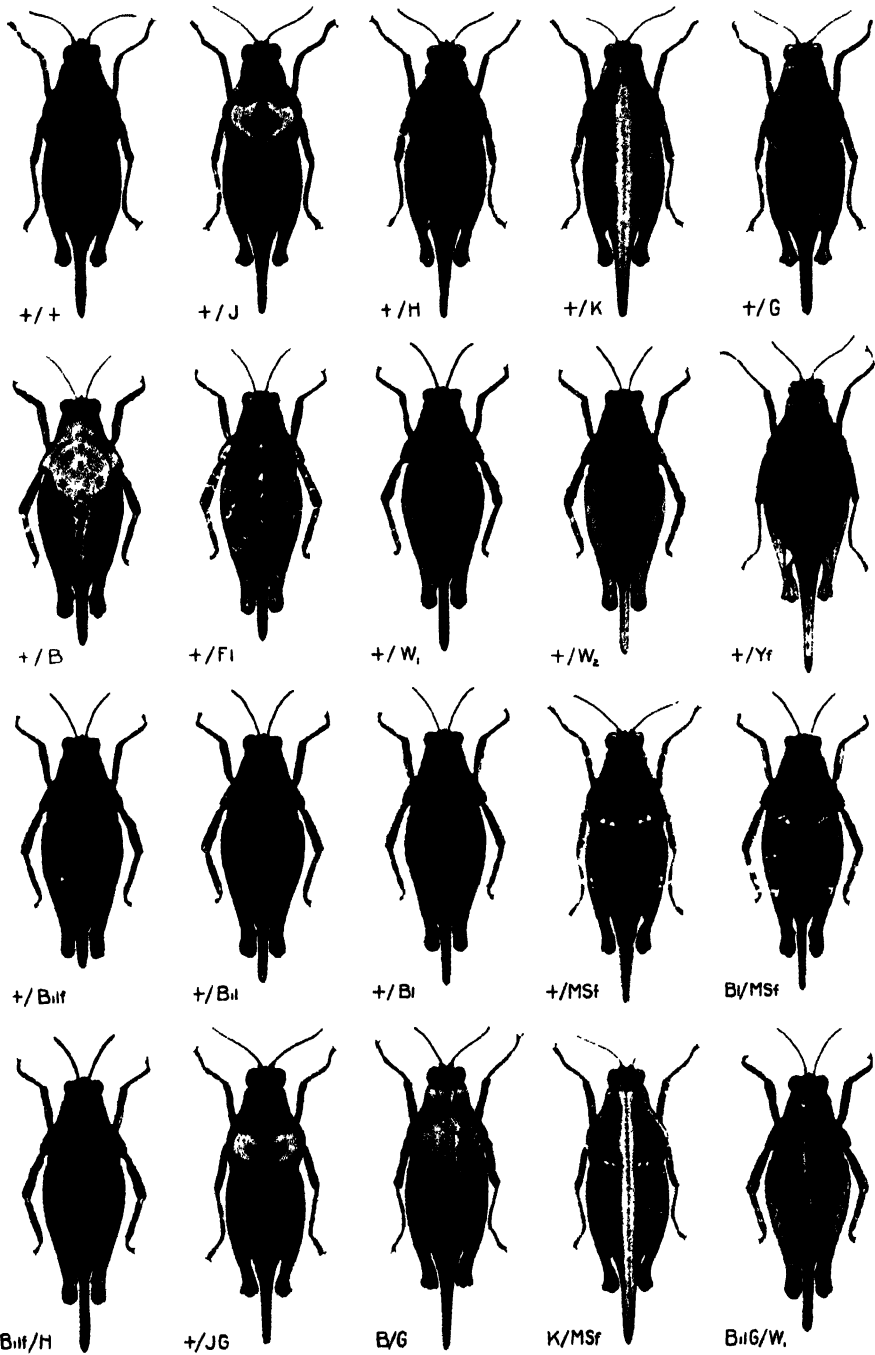
4. *Bilf*. Bilateral line, as in *Bil*, but heavier and extending from anterior end of pronotum. Lines also along upper posterior part of femora of hind legs.

5. *Bl*. Entire pronotum, upper parts of lateral lobes and posterior two-thirds of femora of jumping legs dense black.

## LEGEND FOR PLATE 1

(*Acrydium arenosum* Burmeister)

The mottled gray, recessive, or "wild type" pattern (+/+) and fourteen of the dominant, elementary color patterns are shown in the first fourteen drawings, from left to right. The +/*M Sf* represents two elementary patterns, *M* and *Sf*, which are not indicated separately. The last seven drawings are representatives of the numerous hybrid complexes. S. FRED PRINCE made the first five, the tenth, fourteenth, seventeenth, eighteenth and nineteenth, and RUTH CLENCY made the other ten drawings.





6. *D.* Strong white spots above superior carinae near middle of hind femora.

7. *Fl.* Small, irregularly spaced white flecks over pronotum, lateral lobes, all femora, with general reddish brown background.

8. *G.* Tawny brown stripe along median pronotum.

9. *Gr.* Light, mottled gray over pronotum, lateral lobes and femora of jumping legs. Four small black spots distributed along median carina.

10. *H.* Light orange brown, oblong spot, the width of the pronotum, between the humeral angles.

11. *J.* Nearly clear white spot, with small area of mottled (+/+ ) pattern in center. Occupies the same area as *H*.

12. *K.* Gray white stripe along median pronotum.

13. *M.* One, or more, small, variable, white spots just posterior to the humeral angle, on each side of the pronotum.

14. *My.* A mahogany brown over posterior three-fourths of pronotum.

15. *Myfem.* Reddish brown, as in *T*, of jumping legs, and lines anterior to humeral angle, and reddish tip of pronotum.

16. *R.* Entire pronotum dull yellow. Femora of hind legs marked as if they carried pattern of *W*<sub>2</sub>.

17. *Sf.* One or two small white spots on each femur of jumping legs, in nearly the same position as *D*.

18. *T.* Deep red brown extending all over pronotum and legs. Tips of wings and dorso-lateral sides of abdomen strongly tinged with red.

19. *W.* White to brown fine line along each femur of posterior legs.

20. *W*<sub>1</sub>. Light brown or orange over most of the femora of the jumping legs, and extending as a fine line around the humeral angles, and affecting the tip of the pronotum.

21. *W*<sub>2</sub>. Broad yellow stripe along each femur of hind legs, spreading out over distal two-fifths. Tip of pronotum yellow.

22. *Yf.* Light lemon yellow over distal fourth of each femur of jumping legs.

23. *Yfext.* Similar to *Yf*, but central part of yellow pattern extended.

Two other distinct patterns, one resembling *M*, though with outer edges of pattern extending anteriorly and posteriorly as a fine line along the lateral carinae, and another, (*N*), a dark, reddish brown nearly all over the pronotum, have been bred in small numbers. However, these two patterns do not enter into the data of this paper.

The effects or products of the combinations of the dominant elementary patterns vary greatly. Some combinations leave the constituents, respectively, distinct; others appear to blend, while a few of the dominant complexes are so entirely novel as to be fairly considered hybrid emergents. A long catalog of the manifold effects of patterns on each other and on the

TABLE 1

The following is a list of those dominant color patterns, and the recessive "wild type" which resemble one another in *A. arenosum*, *P. texanus* and *A. eurycephalus* (NABOURS 1929).

<i>A. arenosum</i>		<i>P. texanus</i>		<i>A. eurycephalus</i>
+/+	mottled gray	+/+	recessive	+/+
<i>B</i>	white all over	<i>B</i>	dominant	<i>O</i>
<i>Bl</i>	black all over	$\Theta$	dominant	$\Theta$
<i>D</i>	white spot femora	<i>D</i>	dominant	..
<i>G</i>	brown stripe	<i>P</i>	dominant	<i>G</i>
<i>H</i>	brown spot	<i>H</i>	dominant	<i>H</i>
<i>J</i>	white spot	<i>J</i>	dominant	<i>Y</i>
<i>K</i>	white stripe	<i>K</i>	dominant	<i>K</i>
<i>M</i>	diagonal lines on pronotum	..	dominant	<i>M</i>
<i>N</i>	brown all over	<i>N</i>	dominant	
<i>R</i>	yellow all over	..	dominant	<i>R</i>
<i>Sf</i>	(dom) speckled femora	<i>sf/sf</i>	recessive	
<i>T</i>	red all over	..	dominant	<i>T</i>
<i>W</i> <sub>1</sub>	striped femora	..	dominant	<i>W</i>

results of three or more in combination has been made, but the few mentioned below, and the approximations of the drawings of the plate will have to suffice now. After some experience, the recorders are able, in most cases, even for individuals of considerable complexity, to ascertain by simple examination the composition with respect to the factors for component elementary patterns.

The elementary patterns *D* and *M* seem to retain their individuality, respectively, in whatever combinations found. *Bil* and *Bilf* are always comparatively distinct, except that the femoral lines of *Bilf* are obscured by *B*, *R*, *W*<sub>1</sub>, *W*<sub>2</sub>, *Yf* and *Yfext*, and *T* alters the appearance of *Bil* so that it becomes a light bar on the anterior pronotum. *Sf* is hardly distinguishable with *B* or *W*<sub>1</sub>, but is quite distinct in combination with *W* and *W*<sub>2</sub>. *K* shows as a very narrow, white stripe through the brown of *G*, reddish through *T*, and very white in connection with *B*.

The elementary pattern *G* may be identified in connection with all the patterns. It becomes chalky and lighter with *B*, blacker with *Bl*, very brown with *M*, reddish with *T*, and very little altered by *R*. *J* takes on almost any pattern nearby; it becomes whiter with *B*, darker with *Bl*, yellow with *R*, very red with *T*, and causes *G* to spread out where the two cross. *W* is obscured to some degree by almost any pattern on the femora. *R*, *B* and *Bl* are among the prominent patterns that greatly modify, but seldom obscure others.

#### THE RESULTS OF THE PAIRINGS OF FACTORS FOR ELEMENTARY DOMINANT COLOR PATTERNS

There were two kinds of matings: (1) That in which the parentage of either the male or the female, or both was on record, so that, at least, it

was known of the members of pairs of factors whether they had come in together, or separately from each of the parents; (2) that in which the relations of the members of pairs of factors with respect to parentage was not ascertainable. These were usually in the males and virgin females which had been collected in the field.

In the preparation of the data for table 2 showing percentages of crossing over of pairs of factors, the results from the matings of pedigreed individuals have been followed in estimating the position of segregates, or crossovers, of those of unknown ancestry. Since the pairs of factors, of the unpedigreed parents are so nearly the same in ratios of segregation and crossing over as those respectively of the pedigreed individuals, it is thought that few, if any, mistakes have been made. The following pair of linked genes taken at random, except for the larger numbers involved, indicates the general trend of the percentages to agree. In the matings, from the 476 pairings of the factors *My* and *W*, in the pedigreed males, there were 89 crossovers, or a frequency of 18.7 percent (not shown separately in the table). In the consideration of the offspring of the unpedigreed males bearing these factors, the females to which they were mated having been brought in as nymphs, or otherwise certified as virgin, the pairings were treated in the same way. This was irrespective of the lack of knowledge of the positions of *My* and *W* on the pair of chromosomes, whether they had both come from one parent, or the *My* from one, and the *W* from the other parent. From the 644 pairings of *My* and *W* in these unpedigreed males 148, or 23 percent, were crossovers. These have been added (see table 2) to the data of the pedigreed ones to make a total of 1120 pairings with 237 crossings over, or 21.2 percent, in the males.

Similarly treated, there were 571 pairings of *My* and *W* in the pedigreed females, with 114, or 20 percent, of crossing over, and 1027 pairings, with 142, or 13.8 percent, in the unpedigreed females. The data of the pedigreed and unpedigreed females together constitute 1598 pairings of *My* and *W*, with 256 or 16 percent. There were, therefore, 2718 pairings of *My* and *W* in both the males and females, with an average of 493, or 18.1 percent, of crossing over.

Not only in the example given of the factors *My* and *W* but throughout the table it may be observed that the crossing over of factors in the males, with few exceptions, has been comparable with that of the females. This is in striking contrast with the data from the experimental breeding, especially of *Apotettix eurycephalus*, in which there has been practically no crossing over of factors in the males, and *Paratettix texanus* in which the crossing over of the one factor,  $\Theta$ , with the large group of extremely closely linked factors, or multiple allelomorphs, has been in the males very little over one-half as much as in the females.



TABLE 2

Showing segregation, or crossing over, between members of the pairs of factors for dominant color patterns in *A. arenosum*.<sup>1</sup>

FACTORS	MALES			FEMALES			MALES AND FEMALES		
	NUMBERS OF PAIRINGS	CROSS- OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS- OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS- OVERS	PERCENT OF CROSSOVER
<i>B</i> × <i>Bil</i>				13	0	0.0	13	0	0.0
<i>Bilf</i>				192	0	0.0	192	0	0.0
<i>D</i>	9	5	55.6				9	5	55.6
<i>G</i>	35	12	34.3	76	20	26.3	111	32	28.8
<i>K</i>	29	2	6.9	7	3	42.9	36	5	13.9
<i>My</i>	44	14	31.8	261	112	42.9	305	126	41.3
<i>Sf</i>	9	5	55.6				9	5	55.6
<i>T</i>				145	61	42.1	145	61	42.1
<i>W</i>	29	9	31.0	140	44	31.4	169	53	31.4
<i>Bil</i> × <i>Bl</i>	50	28	56.0	296	136	45.9	346	164	47.4
<i>D</i>	20	8	40.0				20	8	40.0
<i>Fl</i>				40	19	47.5	40	19	47.5
<i>G</i>	159	33	21.1	175	71	40.5	334	104	31.1
<i>H</i>	3	3	100.0				3	3	100.0
<i>J</i>	107	10	9.3	138	4	2.9	245	14	5.7
<i>K</i>	212	34	16.0	270	94	34.8	515	153	26.5
<i>M</i>				108	48	44.4	108	48	44.4
<i>My</i>	357	156	43.7	93	32	34.4	450	188	41.7
<i>R</i>	6	3	50.0				6	3	50.0
<i>Sf</i>	52	10	19.6	108	36	33.3	160	46	28.7
<i>T</i>				54	31	57.4	54	31	57.4
<i>W</i>	429	194	45.2	284	116	40.8	713	310	43.5
<i>W</i> <sub>1</sub>	96	47	48.9	206	96	46.6	312	143	47.4
<i>W</i> <sub>2</sub>	62	20	32.3				62	20	32.3
<i>Yf</i>	53	21	39.6	37	16	43.2	90	37	41.1
<i>Yfext</i>	167	71	42.5	37	17	45.9	204	88	43.1
<i>Bilf</i> × <i>Bl</i>	269	121	44.9	126	58	47.6	395	179	45.3
<i>D</i>	3	3	100.0				3	3	100.0
<i>G</i>	48	3	6.2	168	72	42.8	216	75	34.7
<i>Gr</i>	110	52	47.3	138	67	48.5	248	119	48.0
<i>H</i>	156	16	10.3				156	16	10.3
<i>J</i>	72	0	0.0	6	0	0.0	78	0	0.0
<i>K</i>	235	3	1.3	154	59	38.3	389	62	15.9
<i>M</i>	219	102	46.6	17	9	53.5	236	111	47.0
<i>My</i>	314	148	47.1	327	160	48.9	641	308	48.1
<i>R</i>	228	102	44.7				228	102	44.7
<i>Sf</i>	63	32	50.8	17	6	35.3	80	38	47.5

<sup>1</sup> Explanation: The factors are given in alphabetical order, as they were named when brought in from nature, or resembled those of *P. texianus* or *A. eurycephalus*, respectively. Each factor is shown in its pairings with all the others. Example: *B* with *Bil*, *Bilf*, *D*, etc. Then *Bil* with *Bl*, *D*, etc. Under the columns headed with "crossovers" and "percent of crossover" the term "or segregation" is implied.

TABLE 2 (Continued).

FACTORS	MALES			FEMALES			MALES AND FEMALES		
	NUMBERS OF PAIRINGS	CROSS- OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS- OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS- OVERS	PERCENT OF CROSSOVER
<i>Bl</i> f× <i>Bl</i>									
<i>T</i>				110	47	42.7	110	47	42.7
<i>W</i>	446	209	46.9	518	235	45.2	964	444	46.1
<i>W</i> <sub>1</sub>	408	180	44.1	167	77	46.1	575	257	44.7
<i>W</i> <sub>2</sub>	41	20	48.9	34	17	50.0	75	37	49.3
<i>Yf</i>				84	42	50.0	84	42	50.0
<i>Bl</i> × <i>G</i>	22	12	54.5	86	48	55.7	108	60	55.6
<i>Gr</i>	45	7	15.6				45	7	15.6
<i>J</i>	110	56	50.9	195	90	46.2	305	146	47.9
<i>K</i>	51	29	56.9	769	351	45.6	820	380	46.3
<i>M</i>	7	5	71.4				7	5	71.4
<i>My</i>				190	86	45.3	190	86	45.3
<i>R</i>	9	3	33.3				9	3	33.3
<i>Sf</i>	7	6	85.7				7	6	85.7
<i>T</i>				66	24	36.4	66	24	36.4
<i>W</i>	142	68	47.9	528	133	25.2	670	201	30.0
<i>W</i> <sub>1</sub>	400	191	47.8	391	182	46.5	791	373	47.2
<i>W</i> <sub>2</sub>	101	46	45.5				101	46	45.5
<i>Yf</i>				79	38	48.1	79	38	48.1
<i>D</i> × <i>G</i>	25	15	60.0	90	47	52.2	115	62	53.9
<i>J</i>				59	31	52.5	59	31	52.5
<i>K</i>	29	13	44.8	118	56	47.6	147	69	46.9
<i>My</i>	54	6	11.1	260	27	10.4	314	33	10.5
<i>Sf</i>	54	0	0.0	260	0	0.0	314	0	0.0
<i>W</i>	9	5	55.6	30	14	46.6	39	19	48.7
<i>W</i> <sub>1</sub>	25	10	40.0	90	41	45.6	115	51	44.4
<i>Fl</i> × <i>G</i>	22	10	45.5				22	10	45.5
<i>J</i>	175	92	52.6	55	23	41.8	230	115	50.0
<i>R</i>	64	33	51.6	28	8	28.6	92	41	44.6
<i>W</i>	21	0	0.0				21	0	0.0
<i>W</i> <sub>1</sub>	125	65	52.4	40	22	55.0	165	87	52.7
<i>G</i> × <i>Gr</i>				91	24	26.4	91	24	26.4
<i>J</i>	235	1	0.43	303	101	33.3	538	102	19.0
<i>K</i>	170	18	10.6	109	6	5.5	279	24	8.6
<i>M</i>	27	11	40.7	35	18	51.4	62	29	46.8
<i>My</i>	180	78	43.3	288	137	47.6	468	215	45.9
<i>Sf</i>	52	23	44.2	125	61	48.8	177	84	47.5
<i>T</i>	26	11	42.3	33	13	39.1	59	24	40.7
<i>W</i>	525	250	47.6	364	161	44.2	889	411	46.2
<i>W</i> <sub>1</sub>	149	68	45.6	256	123	48.0	405	191	47.2
<i>W</i> <sub>2</sub>	56	24	42.9				56	24	42.9
<i>Yf</i>	72	29	40.3	222	107	48.2	294	136	46.3
<i>Gr</i> × <i>J</i>				6	3	50.0	6	3	50.0
<i>W</i> <sub>1</sub>	110	40	36.4	19	7	36.8	129	47	36.4

TABLE 2. (Continued).

FACTORS	MALES			FEMALES			MALES AND FEMALES		
	NUMBERS OF PAIRINGS	CROSS- OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS- OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS- OVERS	PERCENT OF CROSSOVER
$H \times K$	199	4	2.0	103	7	6.8	302	11	3.6
$M$	41	24	58.5				41	24	58.5
$My$	148	75	50.7				148	75	50.7
$W$	188	91	48.4				188	91	48.4
$W_1$	3	3	100.0	42	17	40.5	45	20	44.4
$W_2$	103	44	42.7	103	43	41.7	206	87	42.2
$Yf$				42	16	38.1	42	16	38.1
$J \times K$	208	21	10.1	236	91	38.6	444	112	25.2
$M$	21	5	23.8	20	8	40.0	41	13	31.7
$My$	316	142	44.9	542	265	48.9	858	407	47.4
$Myfem$	2	1	50.0				2	1	50.0
$R$	64	34	53.1	15	7	46.7	79	41	51.9
$Sf$	21	9	42.9	78	41	52.6	99	50	50.5
$T$	18	8	44.4	36	14	38.9	54	22	40.7
$W$	601	273	45.4	773	375	48.5	1374	648	47.2
$W_1$	592	283	47.8	468	229	48.9	1060	512	48.3
$W_2$	54	25	46.3	19	10	52.6	73	35	47.9
$Yf$	30	9	30.0	116	48	41.4	146	57	39.0
$Yfext$	13	6	46.2	55	25	45.5	68	31	45.6
$K \times M$	72	32	44.4	32	13	40.6	104	45	43.3
$My$	474	207	43.7	804	357	44.4	1278	564	44.1
$Myfem$	32	16	50.0				32	16	50.0
$R$	183	84	45.9				183	84	45.9
$Sf$	29	13	44.8	145	66	45.5	174	79	45.4
$T$				79	42	53.2	79	42	53.2
$W$	977	430	44.0	1076	499	46.4	2053	929	45.3
$W_1$	176	97	51.1	249	116	46.5	425	213	50.1
$W_2$	134	55	41.0	146	66	45.2	280	121	43.2
$Yf$	12	6	50.0				12	6	50.0
$Yfext$				19	9	47.4	19	9	47.4
$M \times My$	15	4	26.7	118	24	20.3	133	28	21.1
$Sf$	109	7	6.4	385	64	16.6	494	71	14.4
$W$	63	4	6.4	118	19	16.1	181	23	12.7
$W_1$				1	1	100.0	1	1	100.0
$W_2$	87	41	47.1	32	10	31.3	119	51	42.9
$Yf$				9	3	33.3	9	3	33.3
$N$	13	1	7.7				13	1	7.7
$My \times R$	178	83	46.6	4	1	25.0	182	84	46.2
$Sf$	54	6	11.1	371	52	14.0	425	58	13.7
$W$	1120	237	21.2	1598	256	16.0	2718	493	18.1
$W_1$	79	37	46.8	132	55	41.7	211	92	43.6
$W_2$	16	4	25.0	10	3	30.0	26	7	26.9
$Yf$	58	17	29.3	116	56	48.3	174	73	42.0
$Yfext$	217	100	46.1	55	23	41.8	272	123	45.2

TABLE 2 (Continued).

FACTORS	MALES			FEMALES			MALES AND FEMALES		
	NUMBERS OF PAIRINGS	CROSS- OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS- OVERS	PERCENT OF CROSSOVER	NUMBERS OF PAIRINGS	CROSS- OVERS	PERCENT OF CROSSOVER
<i>Myfem</i> × <i>W</i>	19	8	42.1				19	8	42.1
<i>N</i> × <i>W</i>	13	0	0.0				13	0	0.0
<i>R</i> × <i>W</i>	178	66	37.1	13	7	53.9	191	73	38.2
<i>W</i> <sub>1</sub>	64	1	1.6	69	7	10.1	133	8	6.0
<i>W</i> <sub>2</sub>	12	6	50.0				12	6	50.0
<i>Sf</i> × <i>W</i>	57	7	12.3	118	19	16.1	175	26	14.9
<i>W</i> <sub>1</sub>	25	10	40.0	90	41	45.6	115	51	44.4
<i>W</i> <sub>2</sub>				27	9	33.3	27	9	33.3
<i>Yf</i>	9	3	33.3				9	3	33.3
<i>T</i> × <i>W</i>	98	50	51.0	153	58	37.9	251	108	43.0
<i>W</i> × <i>W</i> <sub>1</sub>	62	32	51.6	86	42	48.8	148	74	50.0
<i>W</i> <sub>1</sub>	16	7	43.8	10	3	30.0	26	10	38.5
<i>Yf</i>	78	35	44.9	116	56	48.3	194	91	46.9
<i>Yfext</i>	217	87	40.1	55	15	27.3	272	102	37.5
<i>W</i> <sub>1</sub> × <i>Yf</i>	33	4	12.1	42	5	11.9	75	9	12.0

Reference to the plate and descriptions of the color patterns in the text will give the symbols in the table greater meaning. It does not seem desirable to undertake to occupy the space that would be required to give the results of each mating in detail as was done in the account of the breeding of *A. eurycephalus* (NABOURS 1925).

#### THE DISTRIBUTION OF THE GENES FOR THE DOMINANT ELEMENTARY COLOR PATTERNS

The individuals of the species *Acrydium arenosum* have six pairs of autosomes, and of sex chromosomes, one in the male and a pair in the female. The chromosomes in this species are hardly distinguishable, with respect to number, size, form and arrangement, from those of *Paratettix texanus*, *A. potettix eurycephalus* and others of the grouse locusts (Tettigidae) so far studied (ROBERTSON 1915, 1930). No sex-linked factors have been noted. The genes responsible for the respective, elementary, dominant color patterns of *A. arenosum* are probably pretty well distributed among and along several, or all six of the pairs of the autosomes, as indicated by the data from the pairing of the genes presented in table 2. This is notably different from the situation in *A. eurycephalus* in which all thirteen genes for color

patterns and the lethal are closely linked on one (now known to be the smallest [NABOURS 1931, ROBERTSON 1931]) pair of the autosomes, and *P. texanus*, in which twenty-five or more genes for color patterns are extremely closely linked on one pair, and with only two or three others apparently on other pairs of autosomes.

There are at least three or four linkage groups, and probably more. The genes for the patterns, *B*, *Bil*, *Bilf*, *J*, *H*, *K* and *G* are quite easily located on one pair; *Bl*, *Gr* and *R* on another; *W*<sub>2</sub>, *My*, *W*, *D*, *Sf*, and *M* on still another, while *W*<sub>1</sub> and *Yf* are probably on a fourth pair of chromosomes (see table 2).

Certain of the elementary patterns of *A. arenosum* closely resemble some of those of *P. texanus*, *A. eurycephalus*, and of other species, as indicated in table 1. However, there are considerable differences in the linkage relations among the pairs in the different species. For example, there is no crossing over between *J* and *K* in *P. texanus*, about 7 percent between *Y* (= *J*) and *K* in *A. eurycephalus*, and 25 percent between *J* and *K* in *A. arenosum*; complete linkage between *J* and *P* (= *G*) in *P. texanus*, 4 percent of crossing over between *Y* (= *J*) and *G* in *A. eurycephalus* (NABOURS 1929) and 19 percent between the genes for similar patterns in *A. arenosum*. It should be noted, however, that the patterns are not strictly alike, and they may be severally consequent upon entirely different, and never related, genes.

#### SUMMARY

1. The genes for twenty-four dominant, elementary color patterns of *A. arenosum* are apparently widely distributed among and along most of the six pairs of autosomes, three or more on the one pair, six, or more, on two others, respectively, and two or more on a fourth. The data might easily be interpreted so as to place genes for patterns on the other two pairs of autosomes. None is sex-linked.

2. Crossing over of factors for color patterns occurs in the males in about the same frequency as in the females.

3. Certain pairs of factors for dominant, elementary color patterns in *A. arenosum* differ markedly in linkage relations from those of similar patterns in *P. texanus*, and both of these vary considerably in crossing over values from comparable ones in *A. eurycephalus*.

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# DEFICIENCY AND DUPLICATIONS FOR THE GENE BOBBED IN DROSOPHILA MELANOGASTER

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## INTRODUCTION

The present paper consists of two parts. The first deals with a description of a deficiency involving the locus of the sex-linked gene bobbed. The results obtained have a bearing on the problems of the cytological map of the X chromosome and on the existence of an "inert" region in this chromosome. The second part of the paper is devoted to the study of the interactions between bobbed-deficiency and various duplications covering parts of the region lost in the deficiency. The results may have a bearing on the problem of position effect.

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## ORIGIN OF THE DEFICIENCY

Wild-type males from the "Oregon" stock were treated with a heavy dose of X-rays and crossed to females having attached X chromosomes ( $\overline{XX}$ ). In the offspring of this cross a single male was found (February 1931) which had slightly rough eyes, and short, parallel-sided, and somewhat truncate wings. This aberrant male was crossed to unrelated  $\overline{XX}$  females; all the male offspring exhibited the characteristics of the father. The mutant males were then crossed to wild-type females; the  $F_1$  generation consisted of normal flies. In  $F_2$ , slightly less than one-half of the males showed the mutant characters. It was concluded that the new mutant is a sex-linked recessive. Further tests showed that the new gene is an allelomorph of the previously known sex-linked recessive small-wing ( $s^1$ , located at 54.2 in the X chromosome, see MORGAN, BRIDGES, STURTEVANT, 1925). The new mutant is, therefore, called small-wing-2 ( $s^2$ ). The external effects of  $s^2$  are similar, though perhaps slightly more extreme, than those of  $s^1$ .

An attempt to establish a stock homozygous for  $s^2$  failed, the chromosome carrying  $s^2$  being lethal when present in two doses in females. This was unexpected since  $s^1$  is equally viable in females and in males. Further tests showed, however, that the lethal effect of the chromosome carrying  $s^2$  is not associated with the gene  $s^2$  itself, but with another locus in the



same chromosome. The locus responsible for the lethal effect is that of the gene bobbed (*bb*, 70.0 in the X chromosome). The presence of a bobbed-allelomorph in the chromosome carrying *s<sup>l</sup>2* was ascertained by the following test. The *s<sup>l</sup>2* males were crossed to females homozygous for bobbed. The results are shown in table 1. Females carrying a *s<sup>l</sup>2* X chromosome and a *bb* X chromosome are extreme bobbed in appearance. In fact, they

TABLE 1  
*s<sup>l</sup>2bb<sup>def</sup> ♂ × bb ♀.*

EXTREME BOBBED ♀	WILD-TYPE ♀	EXTREME BOBBED ♂	WILD-TYPE ♂
46	3	2	138

are considerably more extreme than females homozygous for *bb*. It follows that the chromosome carrying *s<sup>l</sup>2* contains an allelomorph of *bb*. For reasons to be presented below this new allelomorph of *bb* is called bobbed-deficiency (*bb<sup>def</sup>*). The X-ray treatment induced, therefore, two independent mutations in the same chromosome, namely *s<sup>l</sup>2* and *bb<sup>def</sup>*. The lethal effect of the *s<sup>l</sup>2-bb<sup>def</sup>* chromosome is to be ascribed to *bb<sup>def</sup>* rather than to *s<sup>l</sup>2*. Indeed, lethal allelomorphs of *bb* are known (bobbed-lethal, *bb<sup>l</sup>*, see MORGAN, STURTEVANT and BRIDGES 1927, STERN 1929a).

The non-appearance of the bobbed-characteristics (short bristles, late hatching from the pupae, sometimes also disarrangement of the abdominal tergites), as well as the viability of the *bb<sup>def</sup>* males, is to be expected. STERN (1925, 1927, 1929a) has shown that bobbed is the only known sex-linked gene having a wild-type allelomorph in the Y chromosome. The effect of *bb<sup>def</sup>* in the males is usually suppressed by the Y chromosome. Only in males having no Y chromosome (XO males), or in males having a bobbed-allelomorph in the Y chromosome, can the bobbed characters be manifested.

In the offspring of the cross shown in table 1 there were three wild-type females and two extreme bobbed males. These classes are due to non-disjunction of the X and Y chromosomes in the males carrying the *s<sup>l</sup>2-bb<sup>def</sup>* chromosome. Such males occasionally produce spermatozoa containing both the X and the Y chromosomes, and spermatozoa containing neither of these chromosomes. The first kind of spermatozoa gives rise to XXY females. In such females the effect of *bb<sup>def</sup>* is suppressed by the wild-type allelomorph of *bb* in the Y chromosome, and such females are wild-type in appearance. The second kind of spermatozoa produces XO males which are extreme bobbed (XO males manifest bobbed in a more extreme form than females homozygous for bobbed, STERN 1927). Non-disjunction of the X-Y pair of chromosomes occasionally takes place in normal males (STERN 1929b), but its frequency is very low. The frequency observed in

our experiment (2.6 percent, table 1) is higher than normal. It seems that the presence of the  $sl^2-bb^{def}$  chromosome increases the frequency of non-disjunction in males.

REDUCTION OF CROSSING OVER PRODUCED BY  
THE  $sl^2-bb^{def}$  CHROMOSOME

Small-wing-2 males were crossed to females homozygous for the combination of the sex-linked recessives known as "X-ple" ( $sc\ ec\ cv\ ct^6\ v\ g^2\ f$ ). The  $F_1$  females were crossed to white males (white is a sex-linked recessive). The results are presented in table 2. The calculated frequencies of crossing over are shown in table 3. The standard frequencies of crossing over in the X chromosome are given in table 3 for comparison (according to BRIDGES and OLBRYCHT 1926, and ANDERSON 1929).

TABLE 2  
 $sc\ ec\ cv\ ct\ v\ g\ 6\ 7\ f$   
1 2 3 4 5  $sl$   $bb-def$  ♀ × w ♂.

MALES ONLY			
0— $sc\ ec\ cv\ ct\ v\ g\ f$	927	6— $f$	1
0— $sl$	1280	7— $sc\ ec\ cv\ ct\ v\ g$	1
1— $sc\ sl$	5	7— $sl\ f$	1
1— $ec\ cv\ ct\ v\ g\ f$	9	3, 6— $sc\ ec\ cv\ f$	1
2— $sc\ ec\ sl$	3	3, 7— $ct\ v\ g$	1
2— $cv\ ct\ v\ g\ f$	2	4, 5— $v\ sl$	1
3— $sc\ ec\ cv\ sl$	2	4, 6— $sc\ ec\ cv\ ct\ f$	4
3— $ct\ v\ g\ f$	3	4, 6— $v\ g\ sl$	1
4— $sc\ ec\ cv\ ct\ sl$	6	5, 6— $g\ sl$	1
4— $v\ g\ f$	11	5, 7— $g$	1
5— $sc\ ec\ cv\ ct\ v\ sl$	7	non-disjunction—w	46
5— $g\ f$	6		
		Total	2320

TABLE 3  
Frequency of crossing over in females heterozygous for the X chromosome carrying  $bb^{def}$ .

INTERVALS	$sc-ec$	$ec-cv$	$cv-ct^6$	$ct^6-v$	$v-g^2$	$g^2-sl^2$	$sl^2-f$
$bb^{def}$	0.6	0.2	0.3	1.0	0.7	0.4	0.2
Standard	6.8	9.7	8.4	14.8	11.2	11.3	
Difference	-6.2	-9.5	-8.1	-13.8	-10.5	-10.7	

The very strong reduction of the frequency of crossing over in the chromosome carrying  $sl^2$  and  $bb^{def}$  is obvious. The following experiments were undertaken in order to determine the frequency of crossing over to the right of the locus of  $f$ ; the intervals lying to the right of  $f$  were not followed in the experiment described above. Small-wing-2  $bb^{def}$  males were crossed

to carnation females (the gene carnation, *cr*, lies at about 8 units to the right of *f*), and the  $F_1$  females were outcrossed to *w* males. Table 4 shows the results. In the presence of *bb<sup>def</sup>* the frequency of crossing over between

TABLE 4  
 $\frac{s^{l^2} \quad bb^{def}}{cr} \quad \text{♀} \times w \text{ ♂ (males only).}$

<i>sl</i>	<i>cr</i>	<i>sl<sup>2</sup> cr</i>	WILD-TYPE	<i>w</i>	TOTAL
849	1282	12	6	35	2184

*sl<sup>2</sup>* and *cr* is 0.9 percent instead of about 10 percent. In another experiment *sl<sup>2</sup> bb<sup>def</sup>* males were crossed to *cr* females, and the  $F_1$  females were outcrossed to *bb Y<sup>bb</sup>* males (*Y<sup>bb</sup>* is an allelomorph of *bb* lying in the Y chromosome; males having *bb* in the X and *Y<sup>bb</sup>* in the Y chromosome manifest the characters of bobbed). The results are shown in table 5. The frequencies of crossing over in the *sl<sup>2</sup>-cr* and the *cr-bb<sup>def</sup>* intervals are 0.5 percent and 0.9 percent respectively. Since *sc* and *bb* are located in opposite ends of the X chromosome, the data presented in tables 2 to 5 show that crossing over is strongly reduced throughout the entire X chromosome.

TABLE 5  
 $\frac{s^{l^2} \quad bb^{def}}{cr} \quad \text{♀} \times bb Y^{bb} \text{ ♂ (males only).}$

<i>sl<sup>2</sup> bb</i>	<i>cr</i>	<i>sl<sup>2</sup> cr</i>	<i>bb</i>	<i>sl<sup>2</sup></i>	<i>cr bb</i>	EXTREME- <i>bb</i>	TOTAL
1385	1861	9	8	20	11	36	3330

A considerable number of flies due to non-disjunction of the X and Y chromosomes appear in the offspring of females heterozygous for *sl<sup>2</sup>* and *bb<sup>def</sup>* (*w* flies in tables 2 and 4, extreme-bobbed flies in table 5). The frequency of non-disjunctional flies varies from 1.1 percent to 2 percent in the different experiments. The normal frequency of non-disjunctional gametes is about 1:1200 (MORGAN, BRIDGES, STURTEVANT 1925). It is concluded that the *sl<sup>2</sup>-bb<sup>def</sup>* chromosome is responsible not only for the reduction of crossing over but also for the increase in the frequency of non-disjunction.

#### SEPARATION OF BOBBED-DEFICIENCY FROM SMALL-WING-2

Since crossing over occurs between *sl<sup>2</sup>* and *bb<sup>def</sup>*, these genes can be separated from each other. The *sl<sup>2</sup> f* male shown in table 2, and the *sl<sup>2</sup> cr* males shown in table 4, carry the left part of the original *sl<sup>2</sup>-bb<sup>def</sup>* chromosome but, presumably, do not carry its right part (containing *bb<sup>def</sup>*). Conversely, the wild-type males shown in table 4 carry the right part of the

original chromosome but do not carry its left part (containing  $sl^2$ ). Homozygous  $sl^2 f$  and  $sl^2 cr$  stocks were established without difficulty. An attempt to establish a stock homozygous for  $bb^{def}$ , but not carrying  $sl^2$ , failed. The lethal effect of the  $sl^2-bb^{def}$  chromosome in double dose in females is clearly due to  $bb^{def}$  and not to  $sl^2$ .

The following experiment shows that  $sl^2$  without  $bb^{def}$  does not influence the frequency of crossing over in the X chromosome. Crosses were made of  $sl^2 cr$  males to  $y cv v f$  females. The  $F_1$  females were crossed to white-eyed males. Among the 1573 males counted in the next generation there were only two white ones; the frequency of non-disjunction in the presence of  $sl^2$ , without  $bb^{def}$ , is not significantly different from normal (see above). Table 6 shows the frequency of crossing over observed in this experiment. The frequencies observed are not significantly different from the standard values.

TABLE 6  
 $\frac{y \text{ } cv \text{ } v \text{ } f}{sl \text{ } cr} \text{ } \varphi \times w \sigma^7 \text{ (males only).}$

INTERVAL	$y-cv$	$cv-v$	$v-sl$	$sl-f$	$f-cr$
Frequency	12.0	20.7	19.6	2.4	8.2

Males carrying  $bb^{def}$  without  $sl^2$ , as well as females heterozygous for  $bb^{def}$ , do not differ from the wild-type in appearance, but their viability and fertility seem to be somewhat below that of the wild-type. The presence of  $bb^{def}$  may be detected phenotypically by making flies heterozygous for other allelomorphs of  $bb$ . Thus, females of the structure  $bb/bb^{def}$  are extreme bobbed (table 1). Likewise, males of the structure  $bb^{def}/Y^{bb}$  show the bobbed characteristics in a more extreme form than males of the constitution  $bb/Y^{bb}$ , but much less extreme than XO males carrying  $bb$ . The allelomorph  $bb^{def}$  seems, therefore, to be completely recessive to the wild-type allelomorphs located in either X or in Y chromosomes; in compounds with other  $bb$  allelomorphs it behaves as the most extreme allelomorph of  $bb$  thus far known.

#### PROBABLE NATURE OF $bb^{def}$

Point-mutations do not, as a rule, affect the frequency of either crossing over or non-disjunction of the chromosomes in which they lie. On the contrary, chromosomal aberrations, such as translocations, inversions, deficiencies, and duplications, frequently affect both crossing over and disjunction. The behavior of  $bb^{def}$  suggests, therefore, that not a point-mutation, or at least not only a point-mutation, but some chromosome-aberration is responsible for its appearance and the manner of its action. Tests

were made for the presence of a translocation associated with  $bb^{def}$ . The results were clearly negative for all the chromosomes. The assumption that an inversion arose in the X chromosome simultaneously with  $bb^{def}$  is improbable, since the presence of  $bb^{def}$  allows some single crossing over to take place in every interval studied; this is not the case in any known inversions.

The mutation from wild-type to  $bb^{def}$  is best interpreted as due to a deficiency, that is, to the loss of a section of the X chromosome carrying the normal allelomorph of bobbed. According to MOHR (1923, 1927, 1929) deficiencies behave as the most extreme allelomorphs of the known recessives whose loci are included in the deficient region. This is the case with  $bb^{def}$  (see above). Like all the other known deficiencies  $bb^{def}$  is lethal when homozygous. All known deficiencies eliminate crossing over in the sections



FIGURE 1.—Chromosomes of females heterozygous for bobbed-deficiency. X, the normal X chromosome; D, X chromosomes carrying the deficiency.

involved (BRIDGES 1917, MOHR 1923, 1927), but the longest ones also decrease the frequency of crossing over in the sections of the chromosome adjacent to the deficient section (LI and BRIDGES 1929, MORGAN, BRIDGES and SCHULTZ 1931). The cytological findings (see below) prove that  $bb^{def}$  is actually a deficiency.

#### CYTOLOGY OF BOBBED-DEFICIENCY

The phenomenon of deficiency was discovered and studied in *Drosophila* by BRIDGES (1917, 1919) and by MOHR (1919, 1923, 1927, 1929). BRIDGES (1917) defined the term “deficiency” to mean “the loss or inactivation of an entire, definite, and measurable section of genes and framework of a chromosome.” The deficiencies studied by BRIDGES and MOHR were short

and invisible cytologically. The assumption that the deficient genes were actually lost from the chromosome was inferred from the genetic data. For this reason BRIDGES admitted the possibility that the deficient genes were not physically lost but merely inactivated.

Some of the more recently described deficiencies have proved to be sufficiently extensive to produce cytologically visible changes in the chromosomes. To this group belong the "v-o" deficiency in mice (PAINTER 1927), several deficiencies in the X chromosome of *Drosophila* (L. V. MORGAN in MORGAN, BRIDGES and STURTEVANT 1928, PAINTER and MULLER 1929, MULLER and PAINTER 1932, DOBZHANSKY 1932a, b), and in *Zea mays* (MCCLINTOCK 1931). PAINTER (1927) proposed the term "deletion" for cases in which the loss of genes is discoverable both genetically and cytologically. Evidently, most if not all of the deficiencies studied by BRIDGES and MOHR are deletions in PAINTER's sense. The existence of two terms for precisely the same phenomenon seems unnecessary.

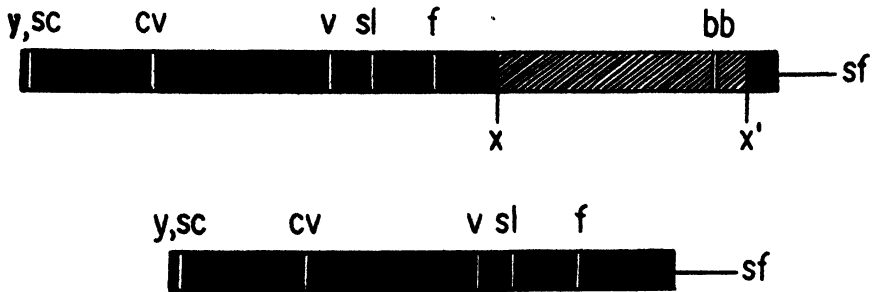


FIGURE 2.—The normal X chromosome (above), and the X chromosome carrying bobbed-deficiency (below). X and X', the points at which the chromosome was broken. The shaded part, the section which is lost in the deficiency. sf, the spindle fibre. Other letters, location of various genes.

Bobbed-deficiency behaves genetically like the deficiencies studied by BRIDGES and MOHR. The deficiency-nature of the allelomorph  $bb^{def}$  was inferred from the genetic data presented above. This deficiency involves the locus of a single known gene, namely that of bobbed. Nevertheless, it is clearly visible cytologically.

Wild-type females were crossed to  $s^{f2} bb^{def}$  males. All the females in the offspring of this cross should be heterozygous for  $bb^{def}$  (barring the possibility of primary non-disjunction). Female larvae were selected, and their nerve-ganglia were fixed in Navashin's solution. Several chromosome-plates were found in this material. Some of them are reproduced in figure 1. In each plate one may see two X chromosomes which are unequal in length. The longer chromosome (X) is presumably the normal X chromosome. The shorter one (D) is the X chromosome deficient for bobbed. The

shorter chromosome is approximately two-thirds the length of the longer chromosome present in the same plate. Hence, in bobbed-deficiency, about one-third of the X chromosome is lost.

Figure 2 represents schematically the structure of the X chromosome in bobbed-deficiency, and also an interpretation of the mode of its origin. The normal X chromosome (figure 2A) was broken in two places ( $x$  and  $x^1$ ), and the section lying between  $x$  and  $x^1$  was lost. The sections lying to the left and to the right of  $x$  and  $x^1$  respectively became united, producing the bobbed-deficiency chromosome (figure 2B). An alternative assumption is that the X chromosome was broken at a single locus (that is, at  $x$ ), and that the section lying to the right of  $x$  was lost. Such an assumption seems, however, improbable since the spindle-fibre is known to be attached to the right end of the X chromosome. The behavior of translocations and deficiencies in *Drosophila* indicates that a chromosome which has lost its spindle-fibre attachment does not behave normally in mitosis, and is therefore eliminated.

#### DUPLICATIONS FOR BOBBED

Several duplications for various sections of the X chromosome were found in the progeny of males treated with X-rays. The origin of these duplications is mostly due to a loss ("deletion," PAINTER and MULLER, 1929) of the middle region of the chromosome, followed by a reunion of the end regions. The resulting chromosomes contain, consequently, only a part of the genes normally located in the X, and consist of two parts corresponding to the left and the right ends of the normal X respectively. Most of the duplications are so small that their addition to the chromosomal complement of males (respectively, females) does not upset the sex balance of the resulting hyperploid individuals. Thus, a duplication female carries two normal X's plus the duplication, that is, a fragment of a third X. Similarly, a duplication male carries one normal X, one Y chromosome, and the duplication.

Six different duplications, covering small portions of the left end of the X chromosome, were selected for the purposes of the present study. They are denoted as "Duplication 101" (described in DOBZHANSKY 1932a), "Duplication 106," 107, 118, 136 (DOBZHANSKY 1932b), and "Duplication 135" (undescribed). The methods used for determination of the loci present in the duplications were outlined in the papers just referred to, and are, therefore, only briefly reviewed here. Individuals are obtained which are homozygous for definite recessive sex-linked genes, and which carry the duplication in question. If the duplication contains the dominant wild-type allelomorphs of the respective recessives, the duplication-carrying individuals fail to manifest the characteristics of the recessives (the duplications contain only wild-type allelomorphs of the sex-linked recessives since

the individuals used for X-rays treatment are usually wild-type males). It was found by this method that duplications 101, 106, 107, 118, and 135 carry the loci of yellow (*y*), scute (*sc*) and silver (*sv*). The duplication 136 carries in addition the loci of kurz (*kz*), broad (*br*) and prune (*pn*).

For the determination of the presence of the locus for bobbed, a slightly different method is used. Females having attached X chromosomes ( $\widehat{XX}$ ), and homozygous for certain sex-linked recessives (the genetic structure of such females is represented in the upper left corner of figure 3), are crossed to *bb/Y<sup>bb</sup>* males (upper right corner of figure 3; *Y<sup>bb</sup>* is an allelo-

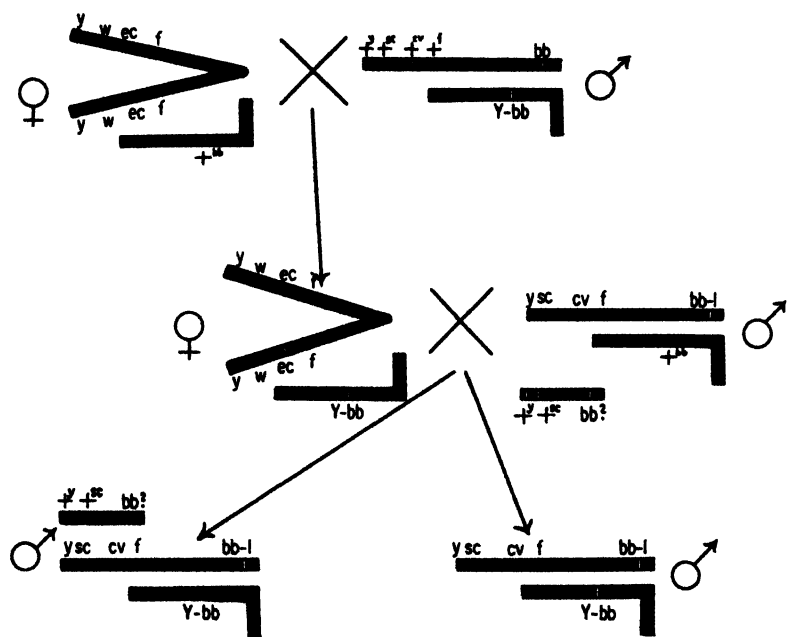


FIGURE 3.—The experimental procedure for testing for the presence of the wild-type allelomorph of bobbed in the duplications. The > shaped chromosome, the attached X chromosomes ( $\widehat{XX}$ ); the rod-shaped chromosome, the X chromosome; the hook-shaped chromosome, the Y chromosome; the short rod-shaped chromosome, the duplication.

morph of bobbed located in the Y chromosome). Females produced in the offspring of this cross are  $\widehat{XX}/Y^{bb}$  (figure 3). Such females are crossed to males carrying the recessives *y*, *sc*, *cv*, *f* and *bb'* in their normal X's, and carrying the duplication to be tested. In the next generation two kinds of males are obtained (lower line in figure 3). Both kinds carry *bb'* and *Y<sup>bb</sup>*, but one kind carries the duplication, and the other is free from it. If the duplication does not contain the locus of bobbed, all males show bobbed in a rather extreme form. If the wild-type allelomorph of bobbed is present in the duplication, the resulting male is non-bobbed. The presence of duplications in a given male is recognized by the suppression of the effects of *y* and *sc*. The results of testing the duplications for the presence of the



locus for bobbed are shown in table 7. One may conclude that duplication 106 does not carry the locus of bobbed, while all other duplications do carry that locus.

TABLE 7  
 $y^2 w^a ec f \widehat{XX} / Y^{bb} \varphi \times y sc cv f bb^1 / Y / \text{duplication } \sigma^1.$

DUPLICATION	$y^2 w^a ec f \varphi$	$w^a ec f \varphi$	$y sc cv f bb \sigma^1$	$cv f bb \sigma^1$	$ec f \sigma^1$
101	274	349	326	..	241
106	190	316	205	67	..
107	96	222	165	..	128
118	115	259	150	..	116
135*	161	314	168	..	134
136*	75	146	81	..	59

\* Duplications 135 and 136 carry  $y^2$  (an allelomorph of  $y$ ) in the fragment. For testing them  $\widehat{XX}$  yellow females were used instead of the  $\widehat{XX} y^2 w^a ec f$  ones.

A different method of testing for the presence of the bobbed-locus in the duplications consists in using females of the structure  $bb/bb^1$ . Such females are, in the absence of duplications, extreme bobbed in appearance (figure 4). If a duplication containing the wild-type allelomorph of  $bb$  is added to the chromosomes of such females (the resulting constitution is  $bb/bb^1/\text{duplication}$ , figure 4), the flies are wild-type. The duplications were also tested by this method; the results were the same as those presented above.

It should be kept in mind, that both methods of testing for the presence of the locus for bobbed in duplications have the following limitation. It is assumed that the duplications may carry either the wild-type allelomorph of bobbed, or not carry the bobbed locus at all. This assumption is based on the fact that the duplications are obtained by treating wild-type (that is, non-bobbed) males with X-rays. However, the X-ray treatment may induce a mutation to bobbed in the duplicating fragment itself. It is easy to see that in such a case the tests may lead to the conclusion that the duplications do not carry bobbed at all. The negative results of the tests are thus not entirely conclusive, while the positive results are more convincing.

#### INTERACTION OF BOBBED-DEFICIENCY WITH BOBBED-DUPLICATIONS

Bobbed-deficiency behaves as a recessive. Its external effects are suppressed by the presence of a single wild-type allelomorph of bobbed. Thus, females of the constitution  $bb^{def}/+^{bb}$ , and males of the constitution  $bb^{def}/Y^+$  (figures 4 and 5), are wild-type. Five of the six duplications described above seem to carry wild-type allelomorphs of bobbed. It is, consequently, justifiable to expect that the presence of these duplications will

suppress the effects of bobbed-deficiency. The experiments show that this expectation is not realised.

Males of the constitution  $bb^{def}/Y^{bb}/\text{duplication}$  were secured. The experiments were so arranged, that males of the constitution  $bb^{def}/Y^{bb}$  were obtained in the same cultures (the experimental procedure applied for getting the  $bb^{def}/Y^{bb}/\text{duplication}$  and the  $bb^{def}/Y^{bb}$  males is similar to that diagrammed in figure 3. The difference consists in using  $y\ sl^2\ bb^{def}/Y^+$  males instead of the  $y\ sc\ cv\ f\ bb^1$  males shown in figure 3). The two kinds of males are exactly alike, except for the presence of the duplication. The inspection of the  $bb^{def}/y^{bb}/\text{duplication}$  males has shown that they are not wild-type, as expected, but are more or less extreme bobbed. Their bristles are short and slender, the abdominal tergites are frequently disarranged, the viability is low. In the absence of bobbed-deficiency the duplications do not produce these characteristics. It seems clear that the wild-type allelomorph of bobbed lying in the duplications fails to suppress bobbed-deficiency.

Some measurements were undertaken for the purpose of obtaining more precise information on the degree of suppression of  $bb^{def}$  by various duplications. Bristles were measured in flies of the constitution  $bb^{def}/Y^{bb}/\text{duplication}$  and  $bb^{def}/Y^{bb}$ . Flies were macerated in a solution of KOH. After washing in water, the flies were transferred in glycerine for clearing. The heads and the thoracal parts of the cleared flies were then isolated, arranged in rows in drops of glycerine on slides, covered with thick cover slips, and flattened as much as possible by means of pressing on the cover slips. The length of the inner vertical, the posterior dorsocentral, and the posterior scutellar bristles was then measured in terms of the units of an eyepiece-micrometer (1 unit being equal to 8.9 mikra). Only one bristle of each kind was measured in each fly. By the "length of the bristle" is meant the distance between the free end of the bristle and its insertion into the theca, irrespective of whether the bristle is absolutely straight or slightly curved.

The results of the measurements are presented in tables 8, 9, and 10. The graphs marked "duplication" indicate the length of the bristles in  $bb^{def}/Y^{bb}/\text{duplication}$  flies; the graphs marked "control" give similar data for the  $bb^{def}/Y^{bb}$  flies from the same cultures. The data for the  $bb^{def}/Y^+$  (wild-type) flies serve as the standard of comparison. For each of the forms studied the mean value (M), its mean error (m), the standard deviation ( $\sigma$ ), coefficient of variation (C), the limits of variation (Lim), and the number of flies measured (n) are given.

The bristles in the  $bb^{def}/Y^{bb}/\text{duplication}$  males are in no case as long as they are in wild-type ( $bb^{def}/Y^+$ ) males. The differences are statistically significant, with a single exception of the dorsocentral bristles in duplica-

TABLE 8.  
*Length of the inner vertical bristle in  $bb^{def}/Y^{bb}/$  duplication and in  $bb^{def}/Y^{bb}$  males.*

	$M \pm m$	$\sigma = \pm$	$C$	$Lim$	$n$
$bb^{def}/Y^+$ (wild-type)	$30.87 \pm 0.20$	1.78	5.8	25-35	81
Duplication 101	$24.71 \pm 0.22$	1.79	7.3	21-35	68
Control 101	$20.00 \pm 0.40$	1.94	9.7	16-24	24
Duplication 106	$21.06 \pm 0.32$	2.36	11.2	16-25	54
Control 106	$20.67 \pm 0.57$	2.82	13.6	17-28	24
Duplication 107	$27.29 \pm 0.22$	1.98	7.3	22-31	81
Control 107	$21.14 \pm 0.50$	2.52	11.8	17-25	25
Duplication 118	$21.48 \pm 0.27$	1.72	8.0	18-27	41
Control 118	$21.14 \pm 0.45$	2.24	10.6	15-24	25
Duplication 135	$27.62 \pm 0.43$	1.74	6.3	25-30	16
Control 135	$23.03 \pm 0.35$	1.36	5.9	21-25	15
Duplication 136	$20.68 \pm 0.30$	1.83	8.9	18-25	37

TABLE 9  
*Length of the posterior dorsocentral bristle in  $bb^{def}/Y^{bb}/$  duplication and in  $bb^{def}/Y^{bb}$  males.*

	$M \pm m$	$\sigma = \pm$	$C$	$Lim$	$n$
$bb^{def}/Y^+$ (wild-type)	$36.84 \pm 0.25$	2.30	6.3	30-40	81
Duplication 101	$32.26 \pm 0.26$	2.13	6.6	26-38	68
Control 101	$25.83 \pm 0.47$	2.29	8.9	22-30	24
Duplication 106	$27.91 \pm 0.40$	2.94	10.5	23-33	54
Control 106	$27.25 \pm 0.74$	3.60	13.2	23-36	24
Duplication 107	$34.62 \pm 0.24$	2.18	6.3	30-40	81
Control 107	$28.18 \pm 0.39$	1.94	6.9	24-32	25
Duplication 118	$28.02 \pm 0.31$	1.96	7.0	24-32	41
Control 118	$28.18 \pm 0.42$	2.08	7.4	22-30	25
Duplication 135	$35.75 \pm 0.39$	1.56	4.4	32-38	16
Control 135	$29.43 \pm 0.37$	1.42	4.8	27-32	15
Duplication 136	$26.68 \pm 0.23$	1.40	5.2	24-30	37

TABLE 10  
*Length of the posterior scutellar bristle in  $bb^{def}/Y^{bb}/$  duplication and in  $bb^{def}/Y^{bb}$  males.*

	$M \pm m$	$\sigma = \pm$	$C$	$Lim$	$n$
$bb^{def}/Y^+$ (wild-type)	$47.91 \pm 0.22$	1.95	4.1	43-52	81
Duplication 101	$38.62 \pm 0.21$	1.71	4.4	35-42	68
Control 101	$33.42 \pm 0.67$	3.31	9.9	29-40	24
Duplication 106	$33.87 \pm 0.42$	3.06	9.0	28-39	54
Control 106	$33.92 \pm 0.98$	4.80	14.2	27-42	24
Duplication 107	$43.34 \pm 0.23$	2.10	4.8	38-48	81
Control 107	$35.62 \pm 0.80$	4.02	11.3	27-44	25
Duplication 118	$35.08 \pm 0.34$	2.18	6.2	30-40	41
Control 118	$35.70 \pm 0.51$	2.54	7.1	27-38	25
Duplication 135	$44.88 \pm 0.44$	1.76	3.9	42-49	16
Control 135	$36.23 \pm 0.42$	1.76	4.9	34-48	15
Duplication 136	$33.00 \pm 0.31$	1.86	5.6	30-37	37

tion 135. It follows that none of the duplications studied suppress completely the effects of bobbed-deficiency. On the other hand, the degree of suppression produced by different duplications is variable. Duplication 135 comes closest to producing a complete suppression. Duplication 107 follows next. Duplication 101 has, in the presence of  $bb^{def}$ , bristles roughly intermediate in length between those observed in wild-type and in  $bb^{def}/Y^{bb}$  males. Finally, duplications 118 and 136 behave very nearly as duplication 106, in spite of the fact that the two former carry the wild-type allelomorph of bobbed, and the latter has no bobbed locus at all.

The effect of duplications on  $bb^{def}$  was also tested by a different method. Females of the constitution  $ClB/y\ s^{l^2}\ bb^{def}$  were crossed to  $y\ bb/Y^{+}$ /duplication males. Some of the non-bar females obtained in the next generation were yellow, and others were non-yellow. The yellow females have the constitution  $y\ s^{l^2}\ bb^{def}/bb$ , and the non-yellow ones the constitution  $y\ s^{l^2}\ bb^{def}/bb$ /duplication. If the duplications suppress the effects of  $bb^{def}$ , the  $y\ s^{l^2}\ bb^{def}/bb$ /duplication females should be non-bobbed. In fact they are more or less distinctly bobbed. The behavior of the different duplications in females is similar to their behavior in males. Thus, duplications 107 and 135 produce a fairly strong, though incomplete, suppression of  $bb^{def}$  in females, while duplication 118 fails to produce marked effects.

The facts presented above apparently leave no escape from the conclusion that the behavior of the wild-type allelomorph of bobbed lying in duplicating fragments is different from the behavior of the same allelomorph lying in the complete X or Y chromosomes. It is worth while to consider here some sources of error, which might conceivably invalidate the above conclusion. One may suppose, for instance, that the presence of the duplications, by altering the genic balance, is *per se* responsible for a decrease (or an increase) of the length of the bristles. This supposition is invalid, since in the absence of bobbed, the duplications (perhaps with the single exception of duplication 136, which makes the bristles thicker and shorter), do not seem to affect the size of the bristles. It is, of course, possible that a very slight effect on the bristle size is produced by the duplications. It remains, however, to be explained why different duplications behave so differently in compounds with  $bb^{+}$  on one hand and in compounds with  $bb^{def}$  on the other.

One may also suppose that the allelomorph  $Y^{+}$  (that is, the wild-type allelomorph of bobbed lying in the Y chromosome) is more effective in suppressing  $bb^{def}$  in males than is  $+^{bb}$  (that is, the wild-type allelomorph of bobbed lying in the X chromosome). If this were so, then the difference in the bristle length which exists between the  $bb^{def}/Y^{+}$  and the  $bb^{def}/Y^{bb}$ /duplication males might be attributed to the higher efficiency of  $Y^{+}$  as compared with  $+^{bb}$ . This supposition is contrary to the following two

facts: (a) different duplications behave differently in the compound  $bb^{def}/Y^{bb}/\text{duplication}$ ; (b) the behavior of the duplications is similar in both sexes.

#### DISCUSSION OF THE RESULTS

##### *The inert region of the X chromosome*

Bobbed-deficiency represents a loss of about one-third of the whole X chromosome. A loss of such a long section of a chromosome might be expected to produce a considerable upset of the genic balance, and, consequently, a somatic effect. This is not the case with bobbed-deficiency. As shown above,  $bb^{def}$  is lethal when homozygous, but has little, if any, effect on the heterozygotes. Females of the constitution  $bb^{def}/+$ , and males

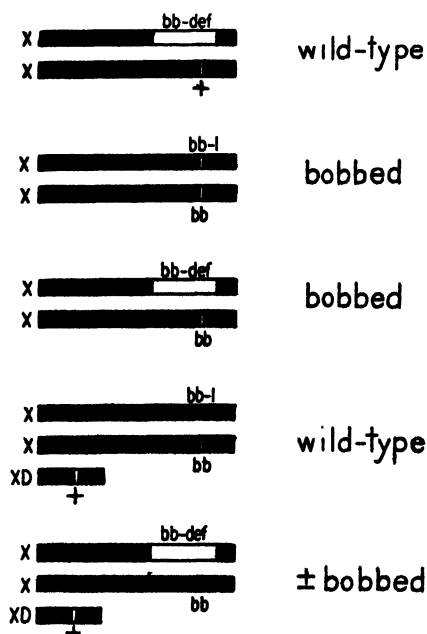


FIGURE 4.—The interaction of the different bobbed allelomorphs in females, X, the X chromosome; XD, the duplication; the white area, the section which is lost in bobbed-deficiency.

of the constitution  $bb^{def}/Y^{+}$  have a somewhat lower viability than wild-type flies, but they are seemingly completely normal in appearance. Deficiencies which are too short to be visible cytologically frequently produce striking dominant somatic effects (MOHR 1923, 1929), and relatively very short deficiencies may be completely lethal in heterozygotes (see, for example, DOBZHANSKY 1930 and 1931). This indicates that the region which is lost in  $bb^{def}$  is less important for development than regions of similar lengths in other chromosomes of *Drosophila*.

PAINTER (1931a, b), MULLER and PAINTER (1932), and DOBZHANSKY (1932b) have shown by combined genetical and cytological studies that

the right one-half or one-third of the X chromosome is made up of a region in which only one gene, namely bobbed, is known to be located. Furthermore, very little, if any, crossing over takes place in this region. PAINTER called this region the "inert region," implying that the number of functional genes per unit of distance is very small in this region. The behavior of bobbed-deficiency constitutes new and fairly conclusive evidence in favor of this view.

The material contained in the inert region of the X chromosome is supposed to be homologous to a section of the Y chromosome. The Y chromosome is also known to be composed of predominantly inert material. As a matter of fact, this assumption seems to have been warranted on the basis of facts which were discovered before anything was known regarding the

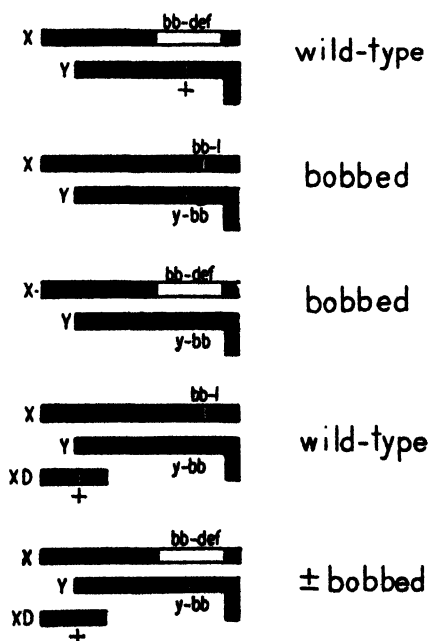


FIGURE 5.—The interaction of the different bobbed allelomorphs in males. Y, the Y chromosome. The significance of other letters is the same as in figure 4.

existence of an inert region in the X chromosome. METZ (1926) in his studies on the spermatogenesis of *Drosophila* has shown that the X and the Y chromosomes do not pair at synaptic stages along their entire lengths, but that only a part of the X undergoes pairing with the Y, the rest of the X remaining unpaired. This indicates that a part of the X is homologous with a part of the Y chromosome.

The behavior of bobbed-deficiency provides further confirmation of the above assumption. Bobbed-deficiency males are normal phenotypically. If some of the genes located in the region of the X which is lost in

$bb^{def}$  have no allelomorphs in the Y chromosome, then  $bb^{def}$  males should not carry these genes at all. It is known, however, that all the known deficiencies so far studied in *Drosophila* are invariably lethal when homozygous. The data of LI (1926) show, furthermore, that individuals homozygous for deficiencies die in very early stages of embryonic development. These data, in the opinion of the present writers, indicate that most, if not all of the genes in *Drosophila* are essential for development, irrespective of whether the known mutations of these genes produce alterations of "superficial" or "fundamental" characters. From this point of view it is improbable that a male which has lost X chromosome genes having no allelomorphs in the Y chromosome would be at all viable, not to speak of being phenotypically normal.

The cytological findings in bobbed-deficiency serve to establish the minimum length of the inert region of the X chromosome. The length of this region is not less than one-third of the length of the X.

*Behavior of the wild-type allelomorph of  
bobbed in duplications*

Bobbed-deficiency is recessive to the wild-type allelomorph of bobbed, provided the latter lies in a normal, unbroken X or Y chromosome. Thus  $bb^{def}/+^{bb}$  females and  $bb^{def}/Y^{+}$  males are wild-type. The situation is different if the wild-type allelomorph of bobbed lies in a fragment of an X chromosome. Five duplications carrying the locus of bobbed were studied. According to their origin they should carry the wild-type allelomorph of bobbed. None of these duplications suppresses the effects of  $bb^{def}$ . Individuals of the constitution  $bb^{def}/Y^{bb}/\text{duplication}$  and  $bb^{def}/bb/\text{duplication}$  are more or less clearly bobbed. Three possible explanations of this phenomenon may be discussed here.

First explanation

The observed facts may indicate that: (a) the development of the wild-type characteristics depends upon the presence of a definite amount of the substance located in the so-called "inert" region of the X, and in the corresponding region of the Y chromosome, rather than upon the action of a specific locus located in these regions; (b) the known allelomorphs of bobbed ( $bb^{def}$ ,  $bb^1$ ,  $bb$ ,  $Y^{bb}$ ) represent losses of varying amount of this substance, which, for the purposes of the present discussion we may call "the bobbed-substance"; (c) the smaller the amount of the "bobbed-substance" present in the germ plasm, the more extreme become the characteristics of bobbed in the adult fly.

None of the duplications studied include the entire inert region of the X chromosome (DOBZHANSKY 1932b). Hence, none of them carries the

amount of the "bobbed-substance" present in the normal X. The duplications include, however, enough of the "bobbed-substance" to produce the wild-type condition in the combinations  $bb^1/Y^{bb}$ /duplication and  $bb^1/bb$ /duplication. Since  $bb^{def}$  represents a longer deficiency than  $bb^1$ , the presence of the duplications is not sufficient to produce the wild-type condition in  $bb^{def}/Y^{bb}$ /duplication and  $bb^{def}/bb$ /duplication flies (figures 4 and 5).

STERN (1929a) has shown that accumulation of the bobbed allelomorphs in the germ plasm results in a gradual approach toward the wild-type condition in the phenotype. Thus, according to STERN, individuals of the constitution  $bb^1/bb^1$  are inviable,  $bb^1/bb$  are extreme bobbed,  $bb^1/bb^1/Y^{bb}$  are less extreme,  $bb^1/bb^1/Y^{bb}/Y^{bb}$  are still less extreme,  $bb^1/bb/Y^{bb}$  are close to wild-type, and  $bb^1/bb/Y^{bb}/Y^{bb}$  are not distinguishable from wild-type. It is easy to see that these results of STERN harmonize perfectly with the interpretation that all bobbed allelomorphs are deficiencies.

STERN discovered, however, another fact which is contradictory to our first explanation. The extreme bobbed allelomorph, known as bobbed-lethal ( $bb^1$ ), was repeatedly observed to revert to a less extreme allelomorph ( $bb$ ), and directly to wild-type. This fact is difficult to reconcile with the assumption that  $bb^1$  is a deficiency.

### Second explanation

The wild-type allelomorph of bobbed is one of the frequently mutating loci. Spontaneous mutations from wild-type to various bobbed allelomorphs are rather common. Though the frequency of mutations at the bobbed locus under the influence of X-rays is unknown, it is not unreasonable to suppose that this frequency is high. The origin of the duplications is due to the breakages caused by X-ray treatment. It is, then, possible that mutations from wild-type to weak allelomorphs of bobbed were induced in the duplications at the time of their origin. This would explain the behavior of the duplications in combinations with  $bb^{def}$ .

This explanation meets with a difficulty, for every one of the five duplications studied needs to be supposed to carry a bobbed allelomorph induced by X-rays. Even if the mutation rate of the bobbed locus is much higher than that for any other known gene, it is very improbable that five duplications would by chance carry such mutations. If the phenomena observed are to be accounted for by mutations at the bobbed locus, an additional assumption is necessary, namely that the occurrence of a breakage in the chromosome strongly increases the probability of mutations taking place in the same chromosome. Such a possibility is, of course, not to be disregarded on *a priori* grounds. There exist, indeed, some facts which argue in favor of such possibility. Translocations very frequently carry lethals,



or mutations producing visible effects, the loci of which are associated with the loci at which the chromosomes were broken (MULLER and ALTENBURG 1930, DOBZHANSKY 1930, 1932c). STERN and OGURA (1931) observed mutations at the bobbed locus which arose simultaneously with translocations involving the X and the Y chromosomes.

### Third explanation

The effect of the wild-type allelomorph of bobbed on development may depend upon its structure as well as upon its position in the chromosome. The loss of the middle part of the X chromosome involves a removal of the material normally located in the vicinity of bobbed, and establishing an association between the locus of bobbed and other loci lying normally far from bobbed. The behavior of the wild-type allelomorph of bobbed in the duplications may, thus, be accounted for by "position effect."

The phenomenon of position effect was discovered in *Drosophila* by STURTEVANT (1925, 1928), who demonstrated that two Bar genes lying in the same chromosome produce a stronger effect than two Bar genes lying in opposite chromosomes. The appearance of "mutations" at the loci of breakages in translocations (see above) may be accounted for by position effect as well as by mutation, and in some cases the explanation by position effect is distinctly preferable to that by mutation (DOBZHANSKY 1932c). The behavior of certain other genes, besides bobbed, in duplications also suggests the existence of a position effect (DOBZHANSKY and STURTEVANT 1932).

The second and the third explanations account equally well for the observed behavior of the wild-type allelomorph of bobbed in the duplications. At the present there seems to be no way for distinguishing between these two explanations experimentally. It is perhaps desirable to point out here that these two explanations may not be mutually exclusive. Mutation represents an alteration of the structure of the gene, and may arise without a breakage taking place in the vicinity of that gene. In case of mutation the alteration is permanent, in the sense that the original condition may be regained only by a reverse mutation. In case of position effect the alteration of the functioning of the gene is due to the removal of other genes normally lying in the neighborhood of the gene in question, or to the association with genes lying normally far from it. Hence, position effect should disappear as soon as the normal order of genes is restored. It is possible, however, that the bonds existing between the adjacent genes in the chromosome are so intimate, that the rupture of these bonds may lead to irreversible alterations in the structure of the gene itself. In such a way a permanent alteration of the structure of the gene (mutation) may be brought about by a change in the position of that gene in the chromosome (position effect).

## SUMMARY

1. A deficiency for the sex-linked gene bobbed was found in the progeny of males treated with X-rays. The presence of the deficiency (symbol  $bb^{ds}$ ) decreases the frequency of crossing over, and increases the frequency of non-disjunction of the X chromosomes.

2. The X chromosome carrying the deficiency is about two-thirds as long as the normal X. It follows that the "inert" region makes up at least one-third of the length of the normal X.

3. The deficiency behaves as a recessive to the wild-type allelomorph of bobbed. Thus,  $bb^{ds}/+^{bb}$  females and  $bb^{ds}/Y^{+}$  males are wild-type in appearance. In compounds with other allelomorphs of bobbed ( $bb$ ,  $Y^{bb}$ ) the deficiency produces an exaggeration of the bobbed characteristics.

4. Five duplications for sections of the X chromosome carrying the locus of bobbed are described. According to their origin these duplications should carry wild-type allelomorphs of bobbed.

5. The wild-type allelomorph of bobbed lying in the duplications fails to suppress the effects of bobbed-deficiency. Three possible explanations of this phenomenon are discussed.

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# COMPARATIVE CYTO-GENETIC STUDIES OF TETRAPLOID TOMATOES FROM DIFFERENT ORIGINS<sup>1</sup>

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## INTRODUCTION

Because the tomato is so well adapted to regeneration in somatic tissue, especially in callus tissue, where chromosome doubling takes place occasionally, the artificial production of tetraploid forms is easily accomplished. By decapitating young tomato plants and allowing a wound callus to form on the cut stem, sprouts arise, some of which carry the doubled chromosome number as the result of a nuclear fusion in the callus (WINKLER 1916, JORGENSEN and CRANE 1927, JORGENSEN 1928, LINDSTROM and KOOS 1931).

The sources of our series of tetraploids are fundamentally four in number, but all arose from one or the other of the two tomato species, *Lycopersicon esculentum*, Mill. and *L. pimpinellifolium*, Dunal. While both of these species have twelve pairs of chromosomes, there is a marked difference between them, both cytologically and phenotypically, and the F<sub>1</sub> hybrid shows a small degree of sterility as evidenced by approximately 10-30 percent pollen abortion. The chromosomes of the *esculentum* species have a diameter at the second metaphase of 1.1 microns while the diameter of the *pimpinellifolium* chromosomes is only 0.8 microns. This fact, coupled with their great phenotypic difference, would seem to justify their separation into two species.

## TETRAPLOIDS

Four kinds of tetraploids are to be considered as follows:

1. Tetraploid from haploid *esculentum* (through the diploid).
2. Tetraploid *esculentum*. Standard variety and variety F<sub>1</sub>.
3. *Pimpinellifolium* tetraploids. Red Currant variety.
  - a. From pure *pimpinellifolium* species.
  - b. From unknown *pimpinellifolium* culture.
4. Tetraploids from F<sub>1</sub> hybrids of species cross.
  - a. Red Currant × *ddpprryy* strain.
  - b. Red Currant × variety Model (potato-leaf, dwarf).

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All of these tetraploids were produced by the decapitation-callus method, using petrolatum to cover the cut end of the stem. The percentage of tetraploid sprouts among the four sources was variable, ranging from none to 30 percent, with an average of about 10 percent. Chromosome doubling in the *esculentum* species was more frequent than in *L. pimpinellifolium*.

A brief description of each tetraploid type, coupled with its breeding behavior, will be given before the cytological studies are reported. Measurements of cell, nucleus and pollen sizes are given in a later section of the paper.

#### *Tetraploid from haploid*

From a haploid *esculentum* form (LINDSTROM 1929) which was practically wholly sterile, numerous markedly fertile diploid forms were produced by decapitation. From the latter were derived the tetraploids (figure 1). This type, possessing four identical chromosomes in each of the



FIGURE 1.—Polyploid series from haploid. Tetraploid-diploid-haploid.

twelve sets and accordingly being absolutely homozygous, was typically sturdy and large in all its parts except the fruit. Although it showed about 80 percent normal pollen, it proved to be the least fertile of all the tetraploids. The set of seed was less than 10 percent that of its parental diploid. The interior of the fruits was characterized by hard, woody placental tissue. Three generations of progeny by self-fertilization have given only tetraploids of the parental form with the same low degree of fertility. Ordinarily only one to four fruits are produced by the best plants. This type is cross-sterile with diploid forms.

*Tetraploid from L. esculentum*

Included in the tetraploid series largely for cytological comparison, were two sets of these tetraploids. One arose from a standard pure strain and the other from a variety  $F_1$  (Marglobe  $\times$  dwarf, peach, ovate, compound-cluster). Since tetraploids of this sort have been reported by others, details are unnecessary. Our tetraploids showed medium fertility. Pollen abortion in the original forms was about 20 percent, and seed development about 40 percent normal. Later progenies of the hybrid exhibited more variation in these respects, but only tetraploids resulted from selfing the



FIGURE 2.—*Pimpinellifolium* diploid and tetraploid.

original tetraploids, whether of pure or hybrid origin. The genetic data from this variety  $F_1$  tetraploid will be included in the section on "genetic observations."

*Tetraploid from L. pimpinellifolium*

From a homozygous strain of this fine-leaved, sprawling species (variety Red Currant), a series of tetraploids arose that are reported here for the first time (figure 2). These were characteristically (for tetraploids) larger and sturdier in stem, leaves, flowers and seeds. Pollen counts showed about 20 percent abortive grains. Fruit size was practically the same as in the diploid or perhaps a trifle greater, but not much. Why fruit size in these tetraploids is not appreciably greater is perhaps not according to expectation, but the fact that the seed set is only 30–40 percent normal is very likely the governing factor. These tetraploids also have only tetraploid progeny, with some variation in fertility although none of 90 plants has been completely sterile. This tetraploid form is completely sterile with the diploid species, when used either as the sire or the dam.

Another *pimpinellifolium* tetraploid has already been reported (LINDSTROM 1932) but since its cytological behavior is much the same as the others, no time will be given to it here, except to note that the fourth and

fifth generations from it continue to breed true, not only to the tetraploid condition, but also phenotypically. In the earlier report, it seemed as if there might be some *esculentum* "blood" in this form, but if so, no evidence of it has yet become apparent. It remains highly fertile for the most part,



FIGURE 3.—Species hybrid tetraploid and diploid.

only 3.0 percent of sterile plants being recorded in the fourth and fifth generations. Further tests have verified its complete sterility with both diploid species.

#### *Tetraploids from $F_1$ of species cross*

Because the chromosome (and pollen) sizes of the two species are significantly different it became particularly interesting to investigate the behavior of the hybrid between the two, both genetically and cytologically. Since the details of chromosome association in the diploid hybrid are un-

der investigation by one of the graduate students in the Department of Genetics, no report will be made of this herein.

Tetraploids were derived asexually by the callus method from two  $F_1$  series of diploid plants (figure 3). The Red Currant variety was crossed by a pure strain of *esculentum* carrying the genes *ddpprryy*, as well as by the variety Model with the genes *ddcc* (dwarf, potato-leaf).

The tetraploid  $F_1$  sprouts when grown, exhibited a somewhat intermediate type between the two species, with, however, the same characteristic dominance of the *pimpinellifolium* species in leaf, stalk, pubescence and fruit size that is found in the diploid  $F_1$  plants. The tetraploid forms were, however, typically sturdier and larger in all respects than the diploid  $F_1$ ,



FIGURE 4.—Species hybrid tetraploid (in center) made by crossing two tetraploids.

even in the matter of fruit size although the difference here was slight. They exhibited the complete dominance of the known genes involved, all carried by the *pimpinellifolium* species. The same species  $F_1$  tetraploid was also produced by crossing  $4n$  *L. esculentum* by  $4n$  *L. pimpinellifolium*, giving results identical with the preceding species tetraploid (figure 4).

The  $F_1$  tetraploid plants were exceedingly fertile. Pollen abortion was approximately the same or slightly less than in other tetraploids, namely 10–20 percent. Seed set in comparison with the diploid was about 50 percent normal, the seeds being fully 30–40 percent larger.

The progeny from both of these tetraploid  $F_1$  species crosses may be classed as highly fertile. Among 720 mature plants, only 3.7 percent were partially sterile and 0.4 percent failed to develop fruit with seeds. Prac-



tically all the others set fruit and seeds remarkably well, some being exceedingly fruitful. All progenies consisted only of tetraploid plants. Cytological tests were made of 20 partially sterile plants but all proved to have 48 chromosomes. Pollen counts showed a range of variability from 7 to 90 percent abortion, centering around 20 percent. Many of the plants with the high degrees of pollen sterility were nevertheless very fruitful.

The  $F_1$  species tetraploid, like the other tetraploids, proved to be completely sterile reciprocally with either of its parental diploid species. Numerous crosses with diploids were made. The crossed fruits remained on

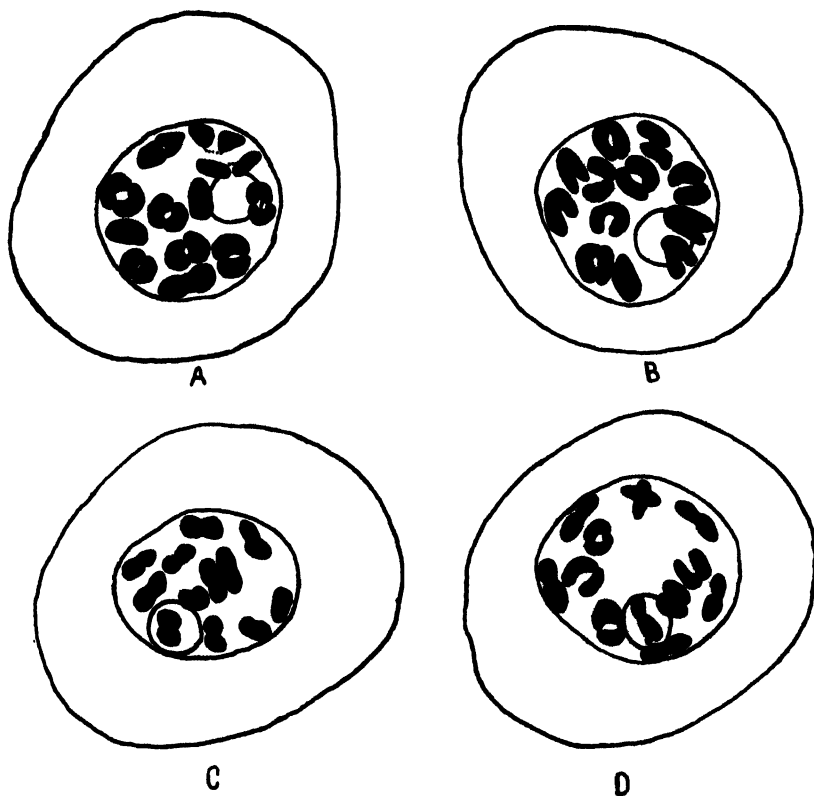


FIGURE 5.—Diakinesis. A—diploid from haploid. B—diploid of *L. esculentum*. C—diploid of *L. pimpinellifolium*. D—diploid of species hybrid.  $\times 2500$ .

the plant, matured and developed a good color and size. But no fertile seeds were produced. The very small, thin, abortive seeds, often in great numbers, seemed to show that there had been a marked stimulation to growth of the ovules, but presumably the embryo failed to develop.

#### CYTOLOGICAL OBSERVATIONS

In general the cytological behavior of all the tomato tetraploids was surprisingly similar, but there were differences which are probably significant.

The material was killed in Allen-Bouin killing fluid and imbedded in paraffin. Sections were cut ten microns thick, and stained in iron alum-hematoxylin. All drawings were made at a magnification of 2500, with the aid of a camera lucida.

In the diploids, at diplotene, there is a partial separation or looping in the paired threads. As diakinesis is approached the chromosomes become much shortened and thickened, and usually the members remain attached at one or more points. In all the diploid cultures under consideration, except the species cross, the members of the bivalents nearly always remain

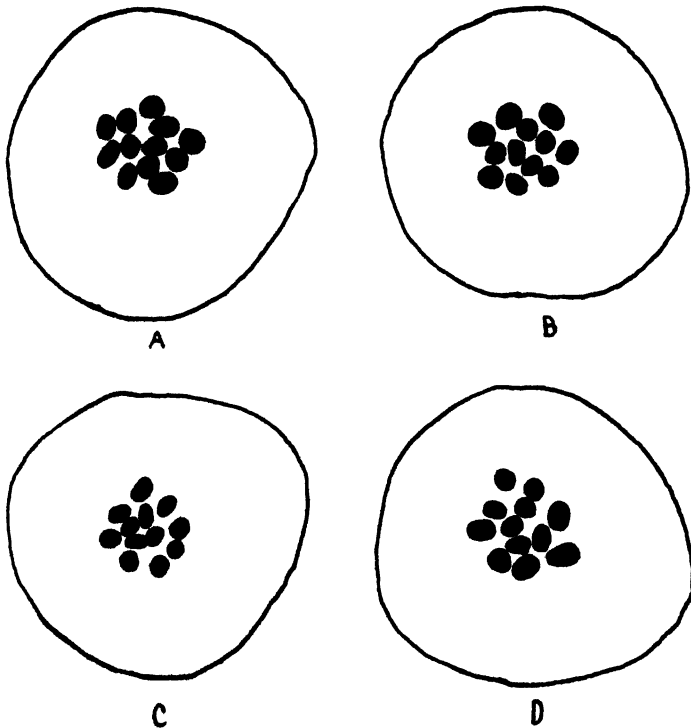


FIGURE 6.—Metaphase I. A—diploid from haploid. B—diploid of *L. esculentum*. C—diploid of *L. pimpinellifolium*. D—diploid of species hybrid.  $\times 2500$ .

connected by at least one point and frequently by two (figure 5, A, B, C). These observations agree in general with those of JORGENSEN (1928), LESLEY (1926), and LINDSTROM and KOOS (1931) on diploid tomato material.

In the diploid of the species cross the association at diakinesis is not as close (figure 5, D). The univalent members of the bivalents are frequently separated at diakinesis, although the figure illustrated does not show this particularly. The pairs are often connected by one end or in loose bivalent rings. At metaphase, however, the bivalents, whether they were separated

or not, reunite into 12 nearly round bivalent chromosomes (figure 6, D), and the anaphase separation is normal.

In the species hybrid, during diakinesis, there is evident, in several of the bivalent pairs, a size difference between the members of the pairs. This might be expected since the average size of the *esculentum* chromosomes is over 30 percent larger than that of the *pimpinellifolium* chromosomes.

A certain general behavior in the tetraploids is apparent. In the prophase, at synapsis, there is a parasynaptic pairing of the four threads at least part of the time. Whether this is true in all cases is not known as yet. At early diplotene there is often visible a four parted thread, which would support the four strand synapsis, for it is very unlikely that the secondary split, presumably taking place at pachytene, would be visible at this stage, especially in material where the chromomeres are not distinct. Counts in early diplotene show approximately 12 twisted and looped threads, though there are sometimes more indicating that synapsis may sometimes be with double and not quadruple chromosomes. However, the number is usually 12 or close to it. From this stage on the tetraploids are considered separately, since individual variations are present in later meiotic stages.

#### *Tetraploid from the haploid*

This tetraploid is the most irregular in chromosome behavior through the stages from diplotene to the first metaphase. At diplotene there is the beginning of a separation of the quadrivalent sets of chromosomes into two parts, usually from the ends. In addition to this separation, there is also a looping within the bivalent elements, probably similar to the diplotene looping in the diploids. Careful observation of this tetraploid shows that at diplotene some of the chromosome threads are undergoing separation while others have not yet started. As early diakinesis is approached the remaining threads commence separation and those which began to split in diplotene have become separated into bivalents. The looping in the individual bivalent threads continues until a condition similar to that in the diploids is reached with these two differences: first, rings are practically never found, the association being end to end or side by side; and second, in some cases the separation is complete and the univalent members of the bivalents become entirely separated (figure 7, A). The diakinesis stages are somewhat irregular because the progress of the individual chromosomes in the cell is not equal. Some will have become separated into bivalents or even univalents, and have become much contracted while others are still in a tetravalent condition and not much shortened. This irregularity continues up to late diakinesis and only then will the separation into bivalents have been largely completed. Even then there

are sometimes groups of four that suggest a tetravalent condition. As metaphase is reached the 24 bivalent chromosomes line up on the equatorial plate and division occurs with regularity. JORGENSEN (1928) observed a condition very similar to this in his tetraploids, but LESLEY and LESLEY (1930) found strikingly different results in a tetraploid arising as a seedling instead of by decapitation. In their tetraploid there were largely

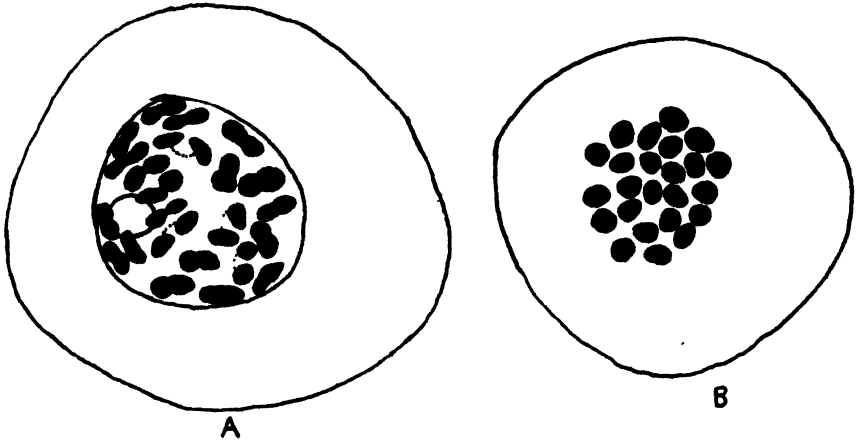


FIGURE 7.—Tetraploid from haploid. A—diakinesis. B—metaphase I.  $\times 2500$ .

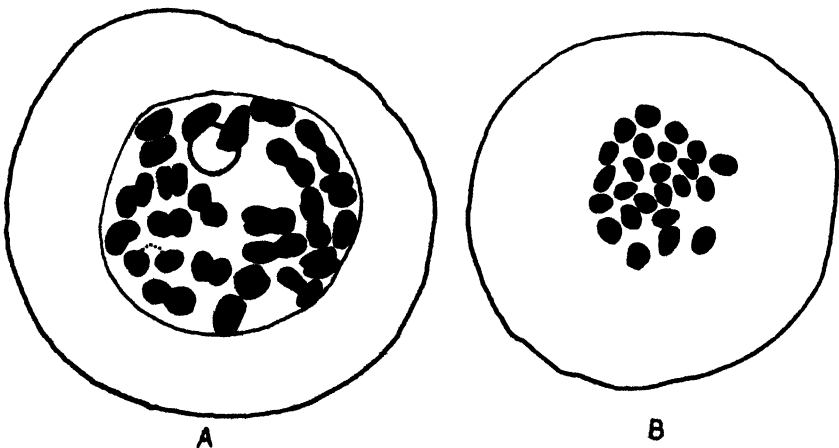


FIGURE 8.—*L. esculentum* tetraploid. A—diakinesis. B—metaphase I.  $\times 2500$ .

tetravalents which persisted through metaphase I causing a very irregular anaphase and an irregular distribution of the chromosomes with consequent irregularities in the second metaphase. None of these irregularities was observed in our material, the behavior being very similar to that observed by JORGENSEN. At metaphase the chromosomes are nearly round and average 1.2 microns in diameter (figure 7, B).

*Tetraploid esculentum*

At diplotene there is evident the same sort of separation as was observed in the tetraploid from the haploid. With the *esculentum* tetraploid, however, all the threads separate at about the same time and subsequent behavior is more regular. In early diakinesis about half of the tetravalents have become separated into bivalents while the rest are still undergoing separation. In mid and late diakinesis there are 24 bivalents, their univalent members being usually connected at one end or at some other point. There are rarely ever bivalent rings, or complete separation of the univalent members. This condition was also observed in the diakinesis of a similar tetraploid form by JORGENSEN (1928) except that he saw occasional tetrasomes persisting to late diakinesis (figure 8, A). Metaphase I shows regularly 24 nearly round bivalents with a diameter of about 1.2 microns (figure 8, B).

*Tetraploid pimpinellifolium*

The diplotene of the tetraploid from the pure species is like the last one described in all respects. At early diakinesis, however, there are usually only bivalents, the separation from tetravalent to bivalent condition having proceeded more rapidly in this form. Throughout diakinesis the univalent members are nearly always connected by one end or side by side, but rarely ever become separated (figure 9, A). Metaphase I consists of 24 bivalents with an average diameter of about 0.9 microns (figure 9, B).

*Tetraploid F<sub>1</sub> of species cross*

The diplotene and early diakinesis stages in this tetraploid are similar to those of tetraploid *esculentum*. By mid diakinesis all the chromosomes are in bivalent condition. The members of the bivalent pairs may be connected at one or more places, and are also frequently separated completely (figure 10, A). However, at metaphase there are 24 bivalents somewhat more variable in size than in any of the other tetraploids (figure 10, B).

During late diakinesis there is frequently evident a difference in size between members of the bivalents identical with that seen in the diploid at the same stage. This suggests that smaller *pimpinellifolium* and larger *esculentum* chromosomes are pairing some of the time. Table 1 includes a brief description of each of the cultures described above.

*Disjunction and anaphase I in tetraploids*

The disjunction and anaphase I are normal in all the tetraploids except that occasionally a pair of chromosomes separates prematurely and proceeds to the poles ahead of the others. About as frequently one or more pairs lag behind the others and arrive at the poles late or not at all. There

is about 20 percent of such irregularity but it is doubtful if this has much to do with the pollen sterility common to the tetraploids, since only about 1 percent of the cells at second metaphase give counts of 23 or 25 chromosomes, and the second division is very regular. This is in agreement with JORGENSEN's observations, but differs sharply from those of LESLEY and LESLEY (1930). They observed irregular second divisions and found varying numbers of chromosomes at second metaphase.

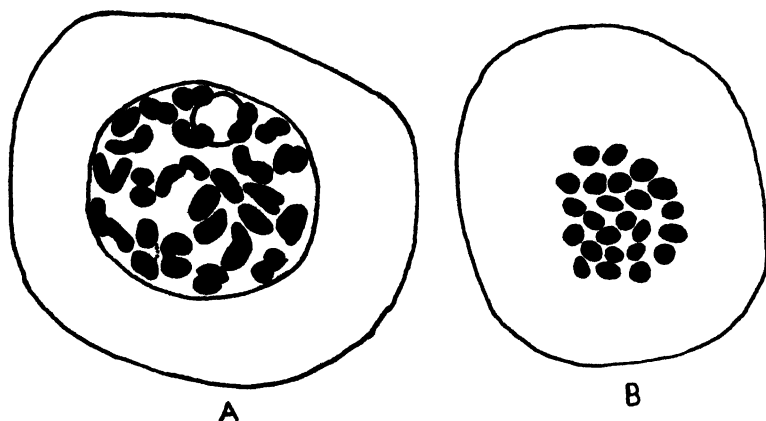


FIGURE 9.—*L. pimpinellifolium* tetraploid. A—diakinesis. B—metaphase I.  $\times 2500$ .

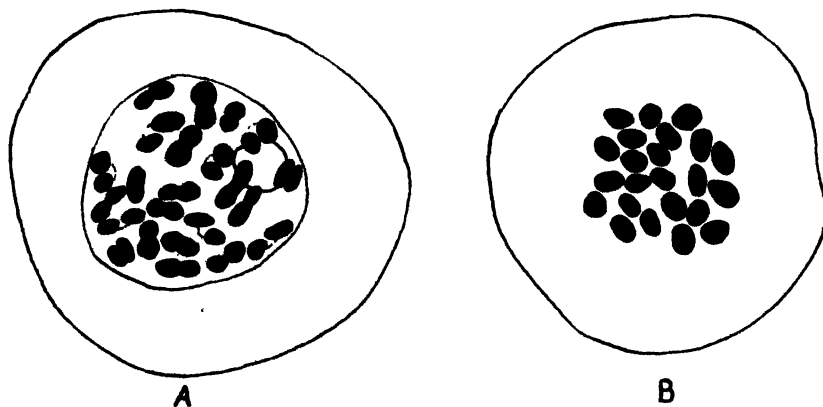


FIGURE 10.—Species hybrid tetraploid. A—diakinesis. B—metaphase I.  $\times 2500$ .

#### MICROMORPHOLOGICAL OBSERVATIONS

Some interesting micromorphological observations were made which are summarized in table 2. At the second metaphase the chromosomes of *L. pimpinellifolium* have a diameter of 0.8 micron, and those of *L. esculentum* a diameter of 1.1 microns, a size difference of over 30 percent. The sizes of 1.2 microns given for the  $F_1$  of the species cross in the table are for the first metaphase where the size is 0.1 to 0.2 micron larger than in the

TABLE 1  
*Cytological behavior of the various cultures.*

CULTURE	DIPLOTENE	EARLY DIAKINESIS	MID DIAKINESIS	LATE DIAKINESIS	METAPHASE I	NATURE OF ASSOCIATION AT MID DIAKINESIS
Diploid from the haploid	Partial separation of the paired threads.	Chromosomes largely connected at ends, forming bivalent rings.	Twelve clear bivalents. All connected. A few flat rings, others connected at one end or throughout length.	Individuals of the bivalents more closely united. No loose formations.	Twelve bivalent units.	No pairs separated. A few connected by one end only. Two-thirds by both ends giving a flat ring. Remainder connected throughout. Association close.
Tetraploid from the haploid	Beginning of separation into bivalents, some looping in bivalent threads.	Partly bivalents and partly in diplotene condition yet. Behavior of individual tetradsomes not regular.	Largely bivalents. Usually some loosely associated tetravalents.	Usually all bivalents. Occasionally one or two groups of four loosely connected.	Twenty-four bivalent units.	Bivalents usually connected at one end or side by side. Frequently the univalent members are entirely separated—about one-sixth of the bivalents. Association fairly close.
Diploid esculentum	Looping of paired threads.	Chromosomes connected at the ends and at other points forming rings and other formations.	Twelve bivalents. Some flat rings—about one-fourth. Largely connected solidly at one end.	Twelve bivalents all closely united.	Twelve bivalent units.	No pairs separated. Association close.
Tetraploid esculentum	Beginning of separation into bivalents. Some looping in bivalent threads.	About half and half tetravalents and bivalents. Progression fairly regular.	Twenty-four bivalents. All closely united, only occasionally a pair stand separated. Union at one end or side by side	Twenty-four bivalents.	Twenty-four bivalent units.	Pairs rarely separated. Association close.
Diploid pimpinellifolium	Looping of paired threads.	Connections at one or more points. Formations irregular.	Twelve bivalents. All univalents connected, nearly always by one end.	Twelve bivalents closely united.	Twelve bivalent units.	No pairs separated. Association close.
Tetraploid pimpinellifolium	Separation into bivalents uniformly preceding. Looping in bivalent threads.	Usually already all bivalents.	All bivalents.	Twenty-four bivalents.	Twenty-four bivalent units.	Only occasional pairs completely separated into univalents. Paired usually end to end. Association close.
Species F <sub>1</sub> diploid	Looping in paired threads.	Loosely connected, usually by ends or one end. Large rings usually formed.	Rings, end to end, and frequently the univalents lie separated. Disparity of size often visible.	Association becoming closer.	Twelve bivalent units.	Occasional pairs separated. Others loosely connected. Association medium. Disparity of size frequently evident.
Species F <sub>1</sub> tetraploid	Beginning of separation into bivalents. Looping in bivalent threads.	Partly tetravalents and partly bivalents. Progression uniform.	Bivalents. Only occasional tetravalents. Disparity in size of univalents often evident.	Twenty-four bivalents usually all mated into bivalents.	Twenty-four bivalent units.	Bivalents sometimes separated into a pair of univalents. Disparity of size often visible. Association medium.

TABLE 2  
Micromorphological characteristics of tomato species.

CULTURE	POLLEN DIAM- ETER	PERCENT DIAM- ETER	SECOND METAPHASE CHROMOSOME DIAMETER	PERCENT DIAM- ETER	CELL SIZE AT DIA- KINESIS	PERCENT SIZE	NUCLEAR SIZE AT DIA- KINESIS	PERCENT SIZE
<i>L. pimpinellifolium</i> —2n	21.6	100	0.8	100	16.7	100	8.9	100
<i>L. pimpinellifolium</i> —4n	27.1	130	0.8	100	21.9	129	12.2	137
<i>L. esculentum</i> —Haploid	25.7	118	1.2	154	13.4	83	7.7	85
Diploid from Haploid	25.7	118	1.1	137	16.5	98	9.0	100
Tetraploid from Haploid	30.0	139	1.1	137	21.8	129	13.1	146
<i>L. esculentum</i> —2n	25.6	115	1.1	137	16.6	99	9.0	100
<i>L. esculentum</i> —4n	28.9	133	1.1	137	21.7	128	12.8	143
F <sub>1</sub> of Species Cross—2n	23.4	108	1.2 MI	154	16.5	98	9.0	100
F <sub>1</sub> of Species Cross—4n*	28.3	131	1.2 MI	154	21.5	127	12.1	137

\* Made by decapitation of diploid of F<sub>1</sub> of Species Cross. All measurements in microns.

second metaphase. Figure 6 shows comparative size differences at the first metaphase.

Pollen diameter was found to vary both with the species and with the number of chromosomes present. *L. pimpinellifolium* had the smallest pollen with a diameter of 21.6 microns, while the tetraploid from the haploid had the largest with a diameter of 30.0 microns. When the chromosome number is increased from 2n to 4n there is a corresponding increase in the size of the pollen grains. In *L. pimpinellifolium* the increase was about 30 percent and in the other forms approximately 20 percent. The F<sub>1</sub> of the species cross has a pollen diameter almost exactly intermediate between the respective parents in both diploid and tetraploid.

The cell and nucleus size of the pollen mother cells at diakinesis was found to vary directly with chromosome number. The sizes were the same in both species. The haploid had the smallest cell and nucleus diameter, 13.4 and 7.7 microns respectively. The diploids with slight variations had cell and nucleus sizes of 16.5 and 9.0 microns respectively, and the tetraploids had cell and nucleus diameters of approximately 21.7 and 12.5 microns respectively.

The micromorphological and gross morphological characters of the cultures have the same trend except in the matter of cell size. Morphologically *L. esculentum* is larger and coarser than *L. pimpinellifolium* in most respects (fruit, seed, trichome, leaf, stem, flowers, and stature). The same relation holds for their pollen and chromosome sizes. In general the tetraploids are larger than the diploids in their morphological characteristics. This is also true of the relative sizes of the pollen grain, as well as of the cell and nucleus at diakinesis.



## GENETIC OBSERVATIONS

The more critical observations in this report center around the genetic behavior of the species cross in which the chromosome sizes are different. Four chromosomes were marked genetically (*Dd*, *Rr*, *Yy* and *Cc* genes). The *pimpinellifolium* species carried all the dominant genes. Only  $F_2$  data are reported, since the tetraploid multiple-recessive form, necessary for a backcross, was not then available. Linkage data are not discussed, since the numerous modifying genes of the *pimpinellifolium* species prevented accurate classification of the *Pp* genes which are linked with the *Dd* genes.

Knowing that the chromosomes of the two species differ cytologically in size, and genetically in many genes, and that they assort from a bivalent condition at diakinesis, one might fully expect a pairing of like chromosomes at this stage. This would give bivalents of the composition *AA* and *aa* for any given set of genes and would result only in *Aa* gametes. Such is not the case, for segregation occurred in every one of the four genes tested (table 3). Evidently unlike chromosomes (from different species) or parts of chromosomes, pair and emerge as bivalents at diakinesis.

TABLE 3  
*Tetraploid  $F_2$  segregation.*

	<i>D</i>	<i>d</i>	<i>S</i>	<i>s</i>	<i>R</i>	<i>r</i>	<i>Y</i>	<i>y</i>	<i>C</i>	<i>c</i>	FERTILE	PARTIALLY STERILE	STERILE
Species hybrid													
RC× <i>dpry</i>	328	5			376	19	382	13			375	21	0
RC× <i>dc</i>	555	23							545	33	335	8	2
Total	883	28			376	19	382	13	545	33	710	29	2
Ratio	32:	1			20:	1	29:	1	17:	1			
<i>Esculentum</i> hybrid	258	4	235	7	224	8					208	26	12
Ratio	64:	1	34:	1	28:	1							

Apparently this unexpected result traces to the behavior of these chromosomes in the prophase stages when they are associated as tetrasomes. Were there merely random assortment of the four chromosomes (or genes *AAaa*) here, a 1 *AA*+4 *Aa*+1 *aa* proportion of gametes would result, giving a 35:1 phenotypic  $F_2$  ratio. The data, however, exhibit a higher percentage of recessives than this in every case.

In preliminary reports of pure *esculentum* tetraploids, the 35:1 ratio seems to be the rule (SANSOME 1931). Our own data on such tetraploids might be considered as showing an approach to the same condition (table 3), although the numbers are relatively small. The *Dd* results cannot be

depended upon with certainty because of the difficulty of classifying when four, three, two, one or no dwarf genes are operating.

The species hybrid tetraploids must be considered as exhibiting a significant departure from this 35:1 ratio, particularly in the *R*, *Y* and *C* genes, where classification is certain. Such departures could be explained on a theoretical random assortment of eight chromatids of any tetrasome, approaching as a limit the 21:1 ratio of dominants to recessives. If the genes were far removed from the spindle fibre attachment, this extreme ratio might well be approached with the help of crossing over. There is no desire at present to stress this point since more critical back-cross data will be available in the future.

The important point is that both genetic and cytological evidence demonstrates that chromosomes which are quite unlike will still pair (and very likely cross over). In a tetraploid such as the one dealt with, we might expect a preferential pairing of the two like chromosomes from each species. Evidently something stronger than the attraction of ordinary Mendelian genes influences chromosome pairing. Apparently the two tomato species, one the domesticated form and the other approximating the wild species, are still closely related through their chromosomes even though they are so different phenotypically. Is it not possible that more fundamental genes (in which the two species are still alike) exercise more attraction for their chromosomes than the genes now differentiating the two forms? Or is there some other agency that determines chromosome pairing?

From his studies of tetraploids in *Lycopersicum*, *Solanum nigrum*, *S. luteum* and *S. nigrum* × *S. luteum*, JORGENSEN (1928) was also forced to a similar conclusion. He maintained that "The capacity for conjugation cannot, as is usually assumed, be considered a reliable measure of the degree of identity of the chromosomes entering meiosis. Other factors than the chromosomes themselves may influence the process of conjugation."

In our tomato tetraploids, particularly the tetraploid from the haploid, where all the four chromosomes in any one set are identical, strict tetrasomic association would be expected, as is the case in *Datura*. While there was evidence of such tetrasomic conjugation in the prophase, the striking thing was the very evident disomic condition at diakinesis. Something outside the chromosomes would seem to be causing the bivalent association at this stage.

Both genetic and cytological evidence then, point to tetrasomic association in prophase and to disomic association at diakinesis. The genetic evidence proves that unlike chromosomes (such as those differentiating the two tomato species) or unlike parts of chromosomes (after any crossing over in the tetrasomes) pair at diakinesis. This is not to be expected on

the usual concepts of chromosome pairing, and suggests that simple gene attraction is not the cause of chromosome pairing at diakinesis in tetraploids.

#### SUMMARY

Tetraploids from four different sources involving the two species of the tomato, *L. esculentum* and *L. pimpinellifolium*, were produced by the asexual, decapitation-callus method. Descriptions, breeding behavior and fertility of these forms are reported.

The least fertile tetraploid was the one derived originally from a haploid form. This type in which all four chromosomes in each of the twelve sets are identical, produced fruit sparsely, and seed development was less than 10 percent normal, although the plant is very sturdy and pollen development excellent.

The most fertile tetraploid was the hybrid between the two species. The pure *esculentum* and pure *pimpinellifolium* tetraploids were intermediate in fertility.

All the tomato tetraploids reported were strikingly similar in two important respects. First, all bred true to the tetraploid condition. Diploids or triploids were not produced. Second, all showed complete, reciprocal sterility with the diploid forms of either species. Being phenotypically different from their parental types, being self-fertile and cross-sterile with all diploids, they offer the possibility of an origin for new tomato species.

Cytologically, all the tetraploids were surprisingly similar in their basic chromosome behavior, a fact which was not expected. At metaphase they usually exhibit 24 bivalents, the tetravalent condition in early prophase having largely disappeared by late diakinesis and metaphase I. There is a difference in the rate of change from tetravalents to bivalents in the various tetraploids.

Disjunction of the bivalents at anaphase was normal, except that occasionally a pair of chromosomes separates prematurely and proceeds to the poles ahead of the others or lags. This apparently does not disturb the end results for less than 1 percent of the cells at second metaphase had more or less than 24 chromosomes. The second division was very regular.

Chromosome size at second metaphase was different in the two species, the *esculentum* chromosomes being 1.1 microns and the *pimpinellifolium* chromosomes 0.8 micron in diameter. There was cytological and genetic evidence that unlike-sized chromosomes in the species cross pair in diploids and tetraploids. Chromosome association in the diploid of the species cross was slightly looser than in ordinary diploids.

The size of the pollen grains varied with the species as well as with total chromosome number. Pollen-mother-cell and nuclear sizes, however, were approximately the same for all diploids; and the tetraploids were all alike but were about 40 percent larger.

Genetically four different chromosomes were tested in the species hybrid tetraploid. All the four genes concerned showed segregation, the  $F_2$  ratios, however, being less than the 35:1 ratio expected on the random assortment of the four chromosomes in any one set. The higher percentage of recessives fits the hypothesis of a more or less random assortment of eight chromatids in any tetrasome.

The genetic and cytological results in this species hybrid tetraploid give some evidence that chromosome pairing is not in general governed by mere gene attraction since the gene differences between the two species were numerous and yet unlike chromosomes apparently paired at diakinesis.

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# SEX-LINKED GENES IN THE FOWL

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## INTRODUCTION

A study of the linkage relations obtaining among certain sex-linked genes of the fowl was begun by the author in 1925, the investigation having been first stimulated by PUNNETT'S summary (1923) and the earlier work of GOODALE (1917), HALDANE (1921) and SEREBROVSKY (1922). After a preparatory detailed survey and several test matings of breeds to discover those containing the most favorable contrast of characters, chief attention was directed to reciprocal crosses between Columbian Plymouth Rocks and Gold Campines.

The former breed carries the dominant sex-linked genes for silver, slow feathering, light shank color, and light eye color, and lacks the barring factor, reputedly confusing in classification. The Gold Campine, on the other hand, carries all the corresponding recessive allelomorphs: gold, fast feathering, dark blue shanks, and dark brown eyes, as well as a number of additional contrasting characters of interest in other connections, such as those involved in down colors and patterns, ear-lobe and egg colors, hen-feathering in the male, etc.

The sex-linkages found were published in a preliminary note (MACARTHUR 1927); the values there reported have been confirmed in substance in the following years.<sup>1</sup> Meanwhile the number of known sex-linked genes has been increased and our knowledge of their localization in the sex chromosome has been extended by a number of other investigators (SEREBROVSKY and WASSINA 1927, WARREN 1928, HERTWIG and RITTERSHAUS 1929, RITTERSHAUS 1930, HERTWIG 1930). As might be expected from the large size of this chromosome (HANCE 1926), the number of sex-linked genes in the fowl is relatively large, seven at least having been determined up to the present, a greater number than is known in either of the autosomal linkage groups.

## CHARACTERS AND FACTORS STUDIED

This report deals chiefly with the linkage relations between four pairs of sex-linked genes, of which the last, controlling light or dark iris color, is so studied for the first time:

<sup>1</sup> The carrying out of this project has been made practicable by the funds and stocks provided through the Department of Biology and the Poultry Department, and by facilities and direct help furnished by C. G. MACARTHUR at Bowmansville.

### 1. *Silver versus gold (S, s)*

These well known factors are easily distinguished in the ground color of the down of even the darkest chicks from the crosses listed below as 3, 4, 5, and 6. More difficulty and delay in classification in the first two crosses caused them to be early discontinued. Though silver is clearly dominant over gold, practically every *Ss* male bore numerous feathers partly gold, due probably to somatic segregation as described by SEREBROVSKY (1926).

### 2. *Slow versus fast feathering (K, k = Sl, sl)*

The rate of feathering is distinguishable with comparative certainty even in  $F_2$  populations by the development attained by the wing quills at hatching and the length of the wing and tail feathers on the tenth day after hatching (WARREN 1925, 1928, 1930).

### 3. *Light versus dark dermal melanin pigmentation in the shank (D, d = Y, y)*

The deep mesodermal pigment beneath the scales is slow developing and in young chicks is often quite masked at first by yellow lipochrome in the skin and for some time or permanently by the black epidermal pigment which occurs especially in the darkest downed chicks (crosses 1 and 2). At or about three months of age, as later rechecks show, the shank color becomes fixed and may be safely, and with few exceptions reliably, classified as either dark (slaty blue or green) or light (white, light blue, or yellow or light green)—(DUNN 1925). Since dark plumage colors blacken the shanks to a troublesome degree, crosses such as 5b and 6b producing pale or restricted black downs, are especially favorable.

### 4. *Light (yellow, orange to bay) versus dark (brownish black) iris color, (Br, br)*

That the main genes controlling this contrast of eye colors are a sex-linked pair is concluded from their typical criss-cross inheritance, as in the reciprocal matings 5 and 6, and from their linkage relations. Such a conclusion has only been gradually won, after a period of confusion and change of views calling to mind the history of dark shank color inheritance.

The range and breed distribution of eye colors in fowls were described by DAVENPORT (1906), who observed that black eye tends to become associated with black plumage. Iris colors were classified histologically by BOND (1919) after the early plan devised for mammals, as: (1) simplex (*pearl*), with no anterior pigment and only a rather opaque stroma to obscure the posterior uveal color in hexagonal cells; (2) duplex, with one layer of anterior pigment, either (a) *yellow* or *red*, the round granules concentrating around connective tissue and in muscle fibres, or (b) *brown* or

*black*, the granules packed in a rich plexus of branching cells; and (3) the doubly pigmented triplex, as in Silky, where a *black* plexus overlies a deeper and separate yellow layer and the melanism is wide spread in corium and other mesodermal tissues. This order was considered in general as the order of ascending dominance or epistasis. In fact, however, it seems that but a few of the published crosses exhibit such autosomal dominance of the brown over the red or yellow eye.

DAVENPORT (1906) noted in  $F_1$  hybrids from Black Minorca ♀ × Dark Brahma ♂ the imperfect dominance of yellow iris over brown. From matings of pearl-eyed Malay or yellow-eyed Buff Orpington hens with Black Orpington cocks BOND (1919) obtained chicks with black down and irides dark at first; but the cockerels became light-eyed at maturity by the gradual withdrawal or atrophy of the surface layer of branching cells containing the brown granules, while the pullets remained dark-eyed. He suggested that this change was sex-limited, but apparently did not make the corresponding reciprocal crosses to exclude the possibility of ordinary sex-linkage. PUNNETT (1923, pp. 157 and 58) found dark eye dominant in Sebright × Hamburgh, but both dark eye and dark shank recessive and apparently sex-linked in the  $F_1$  generations from reciprocal crosses of Brown Leghorn × Black Langshan; also sex-linked was melanism in the Silky × Brown Leghorn matings. Since the main object of this work is to collect, compare, and interpret observations, it is necessary to dissent from PUNNETT'S reference to Spangled Hamburgs and Campines as "dark-eyed and purple-faced" breeds; at least our stock of the former was orange-eyed, and neither breed had the purple in the face, comb and ear-lobe that is found in Silky and some Sebrights. In our crosses the sex-linked type of inheritance has been generally followed.

The chick iris color is evidently somehow influenced by epidermal plumage melanins, and the definitive color, either dark or light, becomes established only with advancing maturity. The presence or absence of the deep brown pigment in the adult iris is not then constantly associated with any particular color in the plumage, shanks, or skin, for in our segregating populations, as in pure breeds of fowls, either the dark or the light eye may be found with either of the skin and all the shank colors and with a great diversity of plumage colors and patterns. Dark-eyed  $F_2$  birds, for instance, range in plumage color, either in the gold or the silver series, from black, through Campine marked, to the flecked, and to the nearly pure white of a very restricted Columbian pattern, and they may have shanks of any of the four main color groups. The breed correlation between black feathers and dark shanks and eyes is also broken in many exceptions, where black fowls have only one, or neither of the latter characters. Conversely, light plumaged  $F_2$  birds with restricted black some-

times have one, the other, both, or neither of them. These crosses do not show what effects, if any, are exerted on iris colors by genes for the various recessive whites, barring, and a number of other colors and patterns. Since the pale birds from crosses 5b and 6b (p. 214) do not develop deep brown eyes, the fundamental color and certain melanin forming and extending factors are possibly prerequisites for the fully dark iris. In this material there was evidently no inhibitor of melanin common to shank and iris mesoderm, but the situations in Silkies and canaries and the known interrelation in mammals between eye and hair colors, hint that common distributing and interacting factors for these pigments in their different sites may be expected here.

### 5. *Li li*

Some observations of the relations of a fifth pair, *Li li*, controlling light or brown in the down have been gleaned from my notes since the literature on the sex-linked down color and pattern factors came to my notice.

#### CROSSES MADE

##### 1. *Brown Leghorn* ♀, *D Br k s*, × *Silver Campine* ♂, *dd brbr kk SS*

The  $F_1$  chicks were all silver and fast feathering, with black pigments so extended and intense as to conceal both parent down patterns, and make the shanks nearly black; the females were brown eyed, the males orange eyed. In the small  $F_2$  population the females segregated for silver and gold; eye and shank color segregations could be followed in only a few favorable cases grown to maturity.

##### 2. *Silver Campine* ♀, *d br k S*, × *R. I. Red* ♂, *DD BrBr KK ss*

The  $F_1$  chicks were either uniform red-brown or campine patterned, the males silver and females gold, and both sexes slow feathering, with bluish-white shanks and slightly darkened yellow eye. In  $F_2$  both sexes segregated for silver and gold, only the females for the remaining factors; silver here appears more frequently linked with dark shanks and dark eyes.

##### 3. *Gold Campine* ♀, *d br k s*, × $F_1$ ♂, $\frac{D Br K s}{d Br k S}$ , ex *R.I. Red*

♀ × *Silver Spangled Hamburg* ♂

The mating producing the male was first made to study the reported sex-linked spangling character (LEFEURE and RUCKER 1923), but this pattern behaved practically like an autosomal dominant.

##### 4. *Columbian Plymouth Rock* ♀, *D Br K S*, × *Silver Campine* ♂, *dd brbr kk SS*

The  $F_1$  chicks were all light to dark gray in down with light silver faces; the females being fast feathering, dark shanked and dark eyed, the males



slow feathering, light shanked and light eyed. The  $F_2$  generation segregated for these three pairs.

5. *Columbian Plymouth Rock* ♀, *D Br K S*, × *Gold Campine* ♂, *dd brbr kk ss*

Both breeds carry black, which is non-extended in the Columbian pattern (*ReRe*), but the Gold Campine (unlike the Silver Campine) strains used differed among themselves in some melanin extension factors (whether *E<sup>me</sup>*, or *tine-atine*—SEREBROVSKY 1926—or others), for in 1926, both in this cross and its reciprocal, 6 below, some pairings produced  $F_1$  chicks of both sexes in two equal and very distinct classes:

a. Dark, slaty (extended), dove-gray to blackish-gray, chicks with light faces (gold in ♀♀, silver in ♂♂), but with no visible down striping.

b. Pale, non-slaty (restricted), all showing a distinct, but more or less modified Campine mottled or 4-striped pattern in the down. The females have less intensely brown eyes than in 5a.

c. New Gold Campine stocks obtained in 1928 from other sources, however, produced when crossed all dark chicks, 51 ♂♂ and 52 ♀♀, with sex distinctions as in 5a.

In crosses 5a, 5b, and 5c the  $F_1$  females are all *d br k s*, the males  $\frac{D Br K S}{d br k s}$ , and the entire  $F_2$  populations segregate for all four of the sex-linked character pairs. Reciprocal back-crosses and numerous  $F_2$  *inter se* matings were made from 5c.

6. *Gold Campine* ♀, *d br k s*, × *Col. P. R.*, ♂

From this cross appeared both

a. Dark, slaty gray chicks with silver faces, and

b. Pale chicks with a Campine-like pattern of brown, black, and light stripes and flecks on the head, cheeks and back. All chicks, whether dark or pale, male or female, were slow feathering and finally developed orange eyes (with occasional faint brown dotting or rays) and light blue or white shanks. In the  $F_2$  only females showed segregation and new crossover classes for the sex-linked factors.

CUTLER (1918) noted, but probably misinterpreted as somehow sex-linked, similar dark and pale classes in hybrids (all males) from Gold Campine ♀ × pheasant ♂.

DATA OBTAINED

A. *Segregation ratios for the different gene pairs*

From these crosses the ratios of the dominant and recessive allelomorphs were separately noted. In some matings as 1, 2, and 6, only females segre-

gated for some or all of the pairs. This fact, and the accumulating losses due to pests and natural deaths, account for the differences in totals. The sex, wherever doubtful, was ascertained by dissection, the sex ratio in  $F_2$  being 269 ♂♂; 289 ♀♀. The other ratios totalled from all the crosses approximate the 1:1 expected, there having been classed: 334 silver: 359 gold; 309 slow feathering: 301 fast feathering; 186 light shanked: 201 dark; and as regards eye color, 191 reddish: 156 brown. No tendency was observed to a sex limitation in iris color or other genes.

*B. Linkage relations between the four pairs  
of sex-linked genes*

Crossover results were taken from either the coupling or repulsion phase or both for all six groupings of the four pairs when they are taken two at a time. The bulk of the data is from the coupling phase in crosses 5 and 6. Tabulated separately in the notes for each phase in each of the crosses the ratios are here merely totalled (table 1). The percentages of crossing over were calculated without correction for differing viabilities of the classes.

TABLE 1

*Showing the number and percentage of crossovers occurring between the series of sex-linked genes.*

FACTOR PAIRS	SEGREGATION		TOTALS	CROSSOVER PERCENTAGES AND THEIR STANDARD ERRORS
	ORIGINAL COMBINATIONS	CROSSOVER COMBINATIONS		
<i>Ss</i> and <i>Kk</i>	520	69	589	$11.7 \pm 1.3$
<i>Ss</i> and <i>Dd</i>	200	177	377	$46.9 \pm 2.6$
<i>Ss</i> and <i>Br br</i>	210	164	374	$43.8 \pm 2.6$
<i>Kk</i> and <i>Dd</i>	206	185	391	$47.3 \pm 2.5$
<i>Kk</i> and <i>Br br</i>	171	157	328	$41.8 \pm 2.7$
<i>Dd</i> and <i>Br br</i>	215	83	291	$27.5 \pm 2.6$

The data indicate linkages of different intensities and significance. Shank colors show so high a percentage of crossing over with silver and gold ( $46.9 \pm 2.6$ ), and also with rate of feathering ( $47.3 \pm 2.5$ ), that assortment is practically free in both cases. Silver-gold and rate of feathering, however, are clearly and closely linked, with only  $11.7 \pm 1.3$  percent of crossing over; this value is much smaller than the 19 percent found by SEREBROVSKY (1922), or the 14 percent of WARREN (1928), and more closely approaches the 10 percent suggested as most likely by HERTWIG and RITTERSHAUS (1929).

Iris colors appear to show a definite though rather loose linkage with both silver-gold and rate of feathering, the crossover values being  $43.8 \pm 2.6$  and  $41.8 \pm 2.7$  percent respectively.

Between shank colors and iris colors another relatively close linkage is evident, for the crossovers are significantly low ( $27.5 \pm 2.6$  percent).

### C. Linkage relations with other sex-linked genes

For reasons stated, barring was intentionally, though perhaps needlessly, avoided in this study; neither spangling nor ear-lobe color (WARREN 1928b) exhibited definite sex-linked behavior; and, though a relatively large number of  $F_2$  chicks were found from crosses 5 and 6 which showed a head spot, and a 4-lined striping, phenotypically resembling those controlled by the *ko* and *st* genes, we have not been able to show in our material that either was sex-linked or associated with sex-linked genes.

The *Li li* factors which inhibit or produce brown in the down of non-black chicks (HERTWIG 1930, HERTWIG and RITTERSHAUS 1929) were not known to us when our notes were taken, but from the first it was clear that the  $F_2$  campine-patterned and other markedly brown chicks were usually, but not invariably, gold and fast feathering. Of 52 such chicks from crosses 5a and 5c, 36 were gold and 16 silver, 35 fast feathering and 12 slow feathering, where equality would be expected, if sex linkage with *li* were not involved. In cross 6a, the 13  $F_2$  chicks which had been classified as campine-like were all (with one possible exception) females, as would be expected in dealing with the sex-linked *Li li* factors. Moreover, the corresponding class of light unstriped chicks, which are among those lacking brown in the down, show an equally marked excess of silver, slow feathering, light shanked and light eyed birds. A rough estimation of *li* linkages from these classes would seem justified since they result from autosomal segregation; the crossing over is about 31 percent between *S* and *Li*, 32 from *K* to *Li*, 16 from *D* to *Li*, and 23 from *Br* to *Li*. Naturally no great stress is to be laid on these figures in the quantitative sense.

### D. Four-point tests

In crosses 5 and 6 the characters are suitably disposed in the parents and the  $F_2$  populations reared large enough for a fairly reliable determination of the number of the single, double, and triple crossovers occurring in the  $F_1$  male (table 3).

Among the 272  $F_2$  fowls, 96 (35.3 percent) represent original combinations, 120 (44.1 percent) single crossovers, 50 (18.4 percent) double crossovers, and 6 (2.2 percent) triple crossing over. The great frequency of double and triple crossovers is attributable to the great cytological length of the sex chromosome and the wide dispersal upon it of the loci studied. The triple crossovers are unexpectedly numerous; since all five *d Br k S* phenotype individuals were entered from a single small population, one of the first to be classified, it seems likely that the value given is too large.

In these tests the crossover percentages approximate those obtained from the whole body of data: *D-Br*, 29.9; *Br-K*, 47.7; *K-S*, 9.9; *Br-S*, 49.6; *D-K*, 44.4; and *D-S*, 46.3.

TABLE 2  
 Showing the frequencies of the different phenotypes segregating in the  $F_2$  generation from  
 $F_1 d br k s \text{ } \varnothing \times F_1 \frac{D Br K S}{d br k s} \text{ } \sigma$ , and among the female progeny from  
 $F_1 D Br K S \text{ } \varnothing \times F_1 \text{ } \sigma$ .

NON-CROSSOVERS	NUMBER	PERCENT
$D Br K S$	48	
$d br k s$	48	35.3
Single crossovers		
$d \mid Br K S$	22	
$D \mid br k s$	8	11.1
$d br \mid K S$	40	29.3
$D Br \mid k s$	40	29.3
$d br k \mid S$	5	3.7
$D Br K \mid s$	5	3.7
Double crossovers		
$d \mid Br \mid k s$	23	
$D \mid br \mid K S$	16	14.4
$d br \mid K \mid s$	3	1.8
$D Br \mid k \mid S$	2	1.8
$d \mid Br K \mid s$	4	2.2
$D \mid br k \mid S$	2	2.2
Triple crossovers		
$d \mid Br \mid k \mid S$	5	
$D \mid br \mid K \mid s$	1	2.2
Total	272	100.0

The numbers in the different classes unfortunately do not settle definitely the sequence of the genes, but silver appears to be more distant than rate of feathering from both  $D$  and  $Br$  loci, and  $Br$  would lie to the left of  $D$ .

#### SEX CHROMOSOME TOPOGRAPHY

Judging from the entire data here supplied, the loci of  $Ss$  and  $Kk$  are somewhat less than 12 units apart. From the average of all the determinations the  $Ss$  locus is separated by at least 44 units and that of  $Kk$  by at least 42 units from  $Brbr$ , and both  $Ss$  and  $Kk$  by still greater distances than these from  $Dd$ . Again,  $Dd$  and  $Brbr$  are about 27.5 units distant. The observed  $D-K$  and  $Br-S$  crossover values agree well with calculated expectations. These and other recent data (HERTWIG 1930) seem to justify reversing the positions of  $K$  and  $S$ , suggested by SEREBROVSKY and WASINA (1927) and WARREN (1928). Such meagre evidence as the material furnishes suggests that the locus of  $Li li$  may be nearer to the center of the chromosome, rather than at the extreme right end (HERTWIG 1930). The

map localization of the four factor pairs might be represented as follows:

<i>D</i>	27.5	<i>Br</i>	42	<i>K</i>	12	<i>S</i>
<i>d</i>		<i>br</i>		<i>k</i>		<i>s</i>

The factors described above are apparently favorably distributed along the chromosome, the *Br br* genes helping to fill in the long empty central portion, and bridge over the wide gap between *D* near one end and *K* and *S* toward the other extreme. But it is still impossible to claim that this or any other present mapping of the sex chromosome is more than tentative, as compared with maps in *Drosophila*, maize, tomato, sweet pea, etc., though this is perhaps the best localized large group of linked genes that is offered in any vertebrate animal.

If we add barring, *B*, and the new genes for head spot, *ko*, and brown-in-down *li* (HERTWIG 1930, RITTERSHAUS 1930), we find uncertainties and inconsistencies also in the localization of these. Combining the data of all authors, and retaining the location of *ko* and *li* proposed by their discoverers, the following linear order and approximate spacings are suggested, merely as a working hypothesis:

<i>B</i>	<i>D</i>	23	<i>Ko</i>	<i>Br</i>	42	<i>K</i>	12	<i>S</i>	17	<i>Li</i>
<i>b</i>	<i>d</i>		<i>ko</i>	<i>br</i>		<i>k</i>		<i>s</i>		<i>li</i>

This provisional arrangement is based upon *one* interpretation of the crossover percentages contributed by the various workers in this field (HALDANE 1921, AGAR 1924, SEREBROVSKY and WASSINA 1927, WARREN 1928, HERTWIG and RITTERSHAUS 1929, HERTWIG 1930, RITTERSHAUS 1930). This data has been reviewed recently in the four last named papers, and further summary is unnecessary.

A critical examination and comparison of the findings shows that where several determinations of a value have been made, their agreement in details is generally good, and often excellent. Yet there are doubtful general points and not a few evidences of internal disharmony in the proposed arrangement as a whole. Such loose linkages have had to be used in the map construction that they provide no very accurate estimate of distances between genes or even of the actual linear order of the genes. Even the point test is no panacea in such a difficulty.

The precise linear relations of *B* and *D* are not sure, since, owing to the masking influence exerted by barring over shank color (WARREN 1928a), their positions and distance are necessarily found only indirectly through third genes. Further and new tests of the linkage of *B* and *D* with the not too distant head spot and iris color genes will doubtless greatly improve the known topography of the left half of the chromosome.

Crossover values between *ko* and *br* have never been taken, but their linkage is theoretically close. When suitable stocks have been found for

making this determination it will be interesting to see how well observation checks with theory in the central portion of the map.

The greatest quantitative discrepancies concern the location of the newer, less investigated genes, particularly *li*. The reported *B-Li* and *D-Li* crossover values are so low—40 and 31 percent respectively—as to appear decidedly anomalous and unlikely, if these genes lie at opposite ends of a chromosome over a hundred units long. *Li* may need to be relocated; in fact the map would fit the present data nearly as well if the *K S Li* section were inverted.

To ascertain the proper order of *K* and *S* in the right half of the chromosome, their linkages will have to be studied with genes that are closer than are *B* and *D*, and perhaps even closer than *Ko* and *Br*.

#### SUMMARY AND CONCLUSIONS

From Columbian Plymouth Rock  $\times$  Gold Campine, and other interbreed crosses, the linkages were studied of the four pairs of sex-linked genes controlling silver or gold in the down and plumage (*S*, *s*), slow or rapid feathering (*K*, *k*), light or dark dermal shank color (*D*, *d*), and orange or brown iris color (*Br*, *br*), the last named pair being new in such studies.

The percentages of crossovers, with their standard errors, found were: *Ss-Kk*,  $11.7 \pm 1.3$ , *Ss-Dd*,  $46.9 \pm 2.6$ , *Ss-Br br*,  $43.8 \pm 2.6$ , *Kk-Dd*,  $47.3 \pm 2.5$ , *Kk-Br br*,  $41.8 \pm 2.7$ , and *Dd-Br br*,  $27.5 \pm 2.6$ .

These values, though not in full agreement with results of a large four-point test, indicate the most likely linear order and the approximate spacings of these genes to be:

<i>Dd</i>	27.5	<i>Br br</i>	42	<i>Kk</i>	12	<i>Ss</i>
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A very provisional mapping of the sex chromosome, on the basis of all the published data and including the loci of barring, *B*, head streak, *ko*, and brown-in-down, *li*, genes, demonstrates a great number of uncertainties and inconsistencies in the proposed arrangement requiring correction by further investigation.

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# THE NATURE OF SIZE FACTORS IN DOMESTIC BREEDS OF CATTLE

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During the past thirty years almost all the investigators who have been interested in size or size inheritance in animals have given some consideration to the nature of the genetic agencies or factors which control size. Even at the present time the question has not been absolutely settled as to whether genes which affect size are general or specific in their nature for the mammalia as a class. Investigators agree that general factors do exist which affect the growth of all parts of the body in the same direction and to proportional degrees. The mooted question is whether or not specific growth factors which affect local areas and units or specific tissues within the individual actually exist. Professor CASTLE (1914 and 1922) from numerous correlation studies gave evidence that factors which control growth in the rabbit are general in their action and affect all parts of the body in the same general direction and to a proportional degree. Moreover, CASTLE found that the lengths of the different bones within an individual are highly correlated, and that all the bone lengths he studied were correlated to a high degree with body weight. In addition to this, CASTLE found for the rabbit that the length of the ears is highly correlated with body weight. These studies seem to show for the rabbit that muscular development is definitely correlated with skeletal development in some manner, and that both are controlled apparently by the same system of general growth factors.

In the studies made by CASTLE, muscle was not considered as a distinct tissue but was included in the total body weight. In 1918 WRIGHT analyzed the data presented by CASTLE in 1914 and came to the conclusion that for the most part, the factors which control bone length are general. However, he concluded that in the development of certain bones and groups of bones there was a slight amount of variation which was controlled by specific growth factors and was consequently independent of general growth factors. In CASTLE's later analyses (1922) this specific action of growth factors was not apparent from his method of analysis. By means of path coefficients and partial correlations WRIGHT (1932) reanalyzed the data on the rabbit given by CASTLE (1914 and 1922), and, in addition, that given by DUNN (1928) on the white Leghorn fowl. WRIGHT concludes that, "In all of these (except the  $F_1$  rabbits) the influence of general size factors preponderates, but the residuals indicate the existence of group factors for



the head apart from general size, of group factors for the fore-limbs and hind-limbs collectively, for the hind-limbs separately and for the wings in fowls, the only case in which two fore-limb measurements were available. Special factors acting on each part separately from the others are also indicated. The genetic differences in the rabbit case were largely in general size, but to a small extent in group and special factors."

The researches of SUMNER (1923) and GREEN (1931) seem to indicate that in the mouse both general and specific factors may affect size. It should be emphasized that in neither the mouse nor the rabbit has there been any selection for differences in types of conformation such as is found in the "dairy" and "beef" types of cattle. It seems reasonable to expect where specific growth factors, which affect muscle diameter (volume), are present they would demonstrate themselves less clearly in species which lack marked contrasting differences in body conformation than in species which have distinct and marked contrasting types of conformation, such as are found in the highly specialized "beef" and "dairy" breeds of cattle, especially since these have been reasonably well purified genetically by selection. For this reason it seems that if breeding tests are not desired, this fundamental question concerning the general or specific nature of growth factors can be studied with profit and advantage in cattle or other domestic animals which present contrasting types of conformation.

In mice, guinea-pigs, rats, rabbits and domestic fowls, it has been clearly demonstrated that size is a quantitative character. Since environmental agencies complicate the problem, all studies in size inheritance in mammals have been somewhat disappointing, especially since no clear-cut size genes have been demonstrated with complete satisfaction. This paper is concerned chiefly with quantitative inheritance. For this reason mutant genes such as dwarfness, which are discontinuous in their effect, will not be considered, as in such cases segregation is clear cut and does not follow laws of quantitative inheritance. Moreover, it should be emphasized that this study makes no attempt to determine whether the factors which control skeletal development are general, group or specific, or a combination of the three. However, from WRIGHT's analysis one should expect the operation of all three with the influence of general factors preponderating.

In a study of growing dairy heifers, ECKLES and SWETT (1919) found that almost any unknown important skeletal measurement can be calculated with a high degree of accuracy from one known skeletal measurement. They also found that height at withers alone was a satisfactory criterion of skeletal development so far as their purpose was concerned. From their investigations it appears that the factors controlling linear

skeletal development in cattle are chiefly general in their action. It should be understood, however, that their studies are not sufficiently extensive to demonstrate the action of group or specific factors which affect skeletal development even if any are involved.

The paper of SWETT, GRAVES and MILLER (1929) which gives an intensive comparative study of the differences in conformation between a highly specialized beef cow and a highly specialized dairy cow, should be mentioned. These investigators came to the conclusion that the skeletal development of the two cows was generally similar, and that the great difference in conformation between the two was caused by the development of extreme "fleshing" on the part of the Angus and the lack of extreme "fleshing" on the part of the Jersey. Unfortunately, the term "fleshing" is not specific. As we understand it the term includes both muscle and fat.

If the problem of size inheritance and conformation is to be solved satisfactorily, it seems that the animal so far as possible should be divided into its component parts of skeleton, muscle, and fat and the inheritance of each part attacked separately. Obviously, many difficulties present themselves in this method. Yet it is a more refined procedure than considering the whole animal as a unit, especially if the investigator can measure muscle and skeletal units with a reliable degree of accuracy.

From the analysis of the development of muscle diameter evidence will be presented in this paper which indicates that some of the genetic agencies which control muscle development are general in their influence. This study is not designed to measure and evaluate the effect of group and specific factors even if some are involved.

Evidence will also be presented which indicates that the genetic agencies affecting linear skeletal development in cattle are different in nature and exhibit a certain degree of independence from those affecting the development of muscle diameter. This indicates that the genetic constitution which affects size and general conformation of an animal may be subdivided, partially at least, into two groups so far as specificity is concerned. One group of genetic agencies is specific for linear skeletal development while the other is specific for the development of muscle diameter. It should be understood that muscles fit the bones so far as linear development is concerned. However, muscles may have a large or small diameter which would, as a consequence, affect the volume (weight). The evidence supporting the preceding statement forms the major part of this study. This does not preclude the possibility that some factors affecting development may influence both muscle diameter and the linear skeleton.

### MATERIAL

The Experiment Station herd of the University of California furnished material for this study. The beef breeds used were Hereford, Shorthorn and Angus. In some cases the  $F_1$  hybrids between these breeds were also included. The dairy breeds were represented by Jerseys, Guernseys, Holsteins and Ayrshires. The Hereford and Angus breeds are similar morphologically, since they are of about the same height and weight, and have about the same muscle measurements. The Shorthorns are slightly taller and heavier than either of the other two beef breeds studied. Of the dairy breeds, the Jerseys and Guernseys are almost identical in build and, as a consequence, their heights, weights, and muscle measurements fall into the same ranges. The Holsteins are distinctly taller than the other dairy breeds. This difference in height is discontinuous with the other breeds. The Ayrshires vary greatly in height at withers (skeletal development), muscle development and, consequently, in weight. There is probably more genetic variability in the Ayrshire breed than in any of the other breeds used in this study.

Approximately three hundred animals furnished data for this study. Of these the greater part were females, but in some of the younger age groups a few steers and bulls were used in the analysis of conformation. In all cases where males were used in the analysis with females, morphological differences in conformation between the sexes caused by the male sex hormone had not become apparent from casual observation.

### ENVIRONMENT AND FEEDING

The growing dairy calves used in this study were not maintained under the same nutritional conditions as the beef calves. Mead (1929) described the method used in feeding the growing dairy calves. In brief it is as follows: During the first two weeks of their life, the calves were fed whole milk. This was gradually replaced by skimmed milk, the amount not to exceed eighteen pounds daily per calf. After they were from ten days to two weeks old the calves were given a concentrate mixture in addition to the skimmed milk and all the alfalfa hay they would consume. The beef calves, on the other hand, remained with their dams until they were about seven months old. After a few weeks of age, they were given alfalfa hay and concentrates. During part of the year the beef calves were allowed to run on pasture. The greatest difference in the two methods was that the beef calves were allowed to remain with their dams, and even provided with a nurse cow in cases where the dam was a poor producer, while the dairy calves were taken from their dams soon after birth and given skimmed milk. After the dairy and beef calves were weaned their treatments were more nearly identical. When good pasture was available no other food was

given to either group. When they did not have access to good pasture they were supplied with hay and concentrates in amounts sufficient to maintain them in good condition.

The dairy cows which tended to lose weight on account of heavy or excessive lactation were fed in such a manner as would enable them to maintain a relatively constant body weight. Lactation in the beef breeds did not change the weight of the dam to any great extent. In collecting the data no correction was made for variation in weight caused by pregnancy. However, the relatively slight errors from this source do not affect the general trend of the data. No corrections were made for variation in weight caused by condition or amount of fat, although this might affect weight to a slight degree. All the cattle used in the study were breeding cows or growing animals of both sexes kept in a relatively uniform condition. Those fitted for either the show ring or the block were not included.

#### METHODS

In this investigation a criterion of linear skeletal development and a criterion of muscle development were established. These were then correlated with each other and with the total body weight. Since the age of the animal is an important factor to be considered, all comparisons of height and muscle development between breeds must be made with individuals of the same age or within limited age groups. For this reason in most all of the comparative analyses between breeds mature cows were used. Since both skeletal and muscle development are taking place simultaneously in young rapidly growing animals, it is extremely difficult to distinguish entirely the effect of skeletal increase from muscle increase on total body weight. However, this may be accomplished if the age limit is exceedingly narrow and the numbers compared are relatively large, in order to eliminate unavoidable environmental variations. Since the number of immature animals was limited, an extensive comparative study of height and muscle between the different breeds for the younger age groups was not undertaken.

In this study the general conformation of each animal is expressed in statistical units. This was accomplished by using the muscle measurement as a numerator and height at withers as the denominator. The mathematical index thus obtained represents in a general way the ratio of muscle diameter to linear skeleton. Different breeds were then analyzed as to conformation by means of this muscle-skeleton ratio, which, from now on, will be referred to as the "muscle-skeletal" index or ratio. Since the muscle-skeletal index becomes practically constant relatively early in the life of the animals, and is independent of the influence of age on weight, it is a

reliable method for expressing differences in conformation between individuals and between breeds. In fact, by using this method of analysis all individuals measured, regardless of age, could be used in comparative studies of breed differences.

#### *a. Height at withers*

As has been stated before, the criterion for skeletal development is height at withers. For the sake of brevity, height at withers will be expressed by the term "height." It should be remembered that ECKLES and SWETT found height a satisfactory method of expressing skeletal development and that in the living animal almost any unknown linear skeletal measurement could be calculated with a high degree of accuracy from this one skeletal length. This measurement is a standard method for expressing skeletal development and, if care is taken, can be made with only a slight error.

#### *b. The round measurement*

On account of the inability to locate definite fixed points it is difficult to obtain accurate non-skeletal body measurements. If no fixed points are used the measurements may vary greatly from time to time and investigators may not be able to duplicate their own or the measurements of others. In order to obtain data as accurate as possible, the muscles in the region of the round were selected for measurement, and two fixed skeletal points were chosen from which to make the measurement. A tape, graduated in centimeters, was placed on the anterior external point of one patella, the first fixed point, and then drawn posteriorly and horizontally about the muscles of the round to a corresponding point on the opposite patella, the second fixed point. The length thus obtained from patella to patella was used as a criterion for the development of muscle diameter. Figure 1 illustrates the method of taking the muscle measurement.

Since the measurement described above has never been used before, some consideration should be given to its appropriateness as a criterion for a muscle measurement. TROWBRIDGE, MOULTON and HAIGH (1918) showed that of all the cuts from the carcass of a beef, the round has the highest percentage of lean (muscle) and that this percentage of muscle to bone and fat remains relatively constant (approximately 80 percent) under the nutritional conditions of maintenance, sub-maintenance and super-maintenance. The fact that the percentage of muscle to bone and fat in the round remains relatively constant even when animals are on different planes of nutrition, together with the fact that the round measurement can be obtained from two definitely fixed points, makes the measurement particularly well adapted as a means of determining the relative amount of muscle in the living animal.

At this point we should give some consideration to the accuracy of the round measurement. In obtaining the measurement, the animal was placed on a level surface, usually a scale, and caused to assume a "normal" standing position, the hind legs being neither too far apart nor too close together, and the hind feet being parallel to a transverse plane through the body. Cow H 154 was measured one hundred times in succession. After



FIGURE 1.—The method used in taking the round measurement.

every third measurement she was forced to change her position, and no attempt was made to have her assume a "normal" position, except that her feet were placed approximately parallel to a transverse plane through the body. They might assume a position close together or far apart, just as she chanced to place herself. The measurements of her round ranged from 93 to 101 centimeters with a mean of  $96.4 \pm .139$  centimeters. The standard

deviation was 2.07 centimeters. Table 1 gives a summary of the measurements taken while she was standing in chance positions. The round of this

TABLE 1  
*Cow H 154, one hundred successive measurements of the round at different positions.*

	<i>f</i>	<i>f.v</i>	<i>d</i>	<i>d<sup>2</sup></i>	<i>fd<sup>2</sup></i>
93	4	372	-3.4	11.56	46.24
94	16	1504	-2.4	5.76	92.16
95	19	1805	-1.4	1.96	37.24
96	20	1920	-0.4	.16	3.20
97	13	1261	0.6	.36	4.68
98	8	784	1.6	2.56	20.48
99	8	792	2.6	6.76	54.03
100	10	1000	3.6	12.96	129.6
101	2	202	4.6	21.16	42.32
	100	9640			430.00
M 96.40 ± .139 S.D.2.073					

same cow H 154 was next measured fifty-seven times in succession after she was made to assume the normal position described above. She was compelled to shift position after every third measurement, but was always forced to assume a "normal" position. The results of this series of measurements are given in table 2. The range is from 94 to 99 centimeters with a mean of  $96.1 \pm .097$ , and a standard deviation of 1.086. The general practice was to take the average of three successive measurements of the round of the animal as the correct one, the animal being made to shift her position between two of the measurements at least. Since in the collection of these data extreme precautions were taken in order to obtain accurate measurements, the actual errors are probably slightly smaller than the one given in table 2.

TABLE 2  
*Cow H 154, fifty-seven successive measurements of the round at "normal" positions.*

	<i>f</i>	<i>f.v</i>	<i>d</i>	<i>d<sup>2</sup></i>	<i>fd<sup>2</sup></i>
94	3	282	-2.1	4.41	13.23
95	14	1330	-1.1	1.21	16.94
96	21	2016	-0.1	.01	.21
97	13	1261	0.9	.81	10.53
98	5	490	1.9	3.61	18.05
99	1	99	2.9	8.41	8.41
	57	5478			67.37
Mean 96.1 ± .097 S.D.1.086					

## HEIGHT IN MATURE ANIMALS

Since this is a comparative study in the various breeds of the differences caused by genetic factors for growth, an analysis of height and its relation to total body weight was made only for mature animals over four years of age. It should be remembered that ECKLES and SWETT (1918) and ECKLES (1920) showed that by the time Jerseys are four years of age they have reached their maximum skeletal development, and after this age they increase slightly in weight only. According to ECKLES, Ayrshires in their skeletal development follow the Jerseys closely, but are heavier in weight. On the other hand, Holsteins and dairy Shorthorns do not complete their skeletal development until they are almost five years of age, and probably do not reach their maximum body weight until about two years after skeletal development ceases. Although a few of the animals included in this mature class of four years or over have not reached their maximum weight and still fewer have possibly not reached their maximum skeletal development, the slight error from this source does not materially affect the data for mature cows nor the conclusions drawn from them.

The heights of all animals four years or more of age are summarized in table 3. On account of their morphological similarity, the Angus cows are summarized with the Herefords, and the Guernseys with the Jerseys.

TABLE 3  
*Height in centimeters of mature cows of all breeds.<sup>1</sup>*

BREEDS	119 118-120	122 121-123	125 124-126	128 127-129	131 130-132	134 133-135	137 136-138	140 139-141	TOTAL	MEAN	S.D.
Hereford	3	3	4						13	122.96 ± .488	2.61
Angus	1	1	1								
Jersey		3	16	16	1				39	126.91 ± .211	1.96
Guernsey		1		1	1						
Ayrshire			3	2	2				7	127.92 ± .622	2.44
Shorthorn			5	3	3	2			13	128.96 ± .615	3.29
Holstein						2	5	2	9	137.83 ± .366	1.63

<sup>1</sup> The original data from which all tables, correlation coefficients, and graphs were made were too massive to include in this paper. The original data are on file at the Brooklyn Botanic Garden and are available on request to any one who wishes to see them.

If height at withers is taken as the criterion for linear skeletal development the Hereford-Angus is less developed than the Jersey-Guernsey group, their height ranging from 118 to 126 centimeters with a mean of  $122.96 \pm .488$  standard deviation 2.61. The Jersey-Guernsey group is distinctly higher with a range in height from 121 to 130 centimeters, a mean



of  $126.91 \pm .211$ , and a standard deviation of 1.96. The limited number of Ayrshires show a mean height of 127.92. The Shorthorns have a range of 125 to 134 centimeters with a mean of  $128.96 \pm .615$ , and standard deviation of 3.29. By this they show considerable variability. The limited number of Holsteins are discontinuously taller than any of the other breeds, since they have a range of 135 to 140 centimeters and a mean of 137.83. Although the total number of mature animals is eighty-one, the numbers are comparatively small when the animals are subdivided and grouped according to breeds. Yet it seems safe to assume that the height found to be the mean for each breed, represents approximately the genetic capacity of the heights of the breeds of the University of California.

With height at withers as a criterion of linear skeletal development, it is evident that there are several different genetic constitutions for height (linear skeletal development) in the different breeds studied. When the Hereford-Angus group is compared with the Jersey-Guernsey group the difference between the mean heights is  $3.95 \pm .531$ . This difference is over seven times the probable error of the difference. When the mean height of the Jersey-Guernsey group is compared with the Shorthorns the difference between the mean is  $2.05 \pm .65$ . This difference is only three times the probable error and is of doubtful significance. However, if larger numbers were observed the difference in height might be found to be significant. The height of the Holsteins is discontinuously greater than that of the Shorthorns. The difference between the means is  $8.87 \pm .715$ . This is more than twelve times the probable error. From this analysis of height it is evident that there are at least three different genetic capacities for height, with some indication of a fourth. The Hereford-Angus group, which has the least linear skeletal development, composes one group. The Jersey-Guernsey group, which is second in linear skeletal development, comprises the second group, while the Holsteins compose the third group. If there is a fourth genetic constitution for height, it is obviously made up of Shorthorns. The differences in height of all breeds, except Holsteins, are of a continuous nature, while that of the Holsteins is clearly discontinuous.

#### ROUND MEASUREMENT IN MATURE ANIMALS

The mature animals of all breeds which were analyzed for height (table 3) were analyzed for round measurements (table 4). For the Jersey-Guernsey group, the round measurement ranged from 83 to 100 centimeters with a mean of  $90.75 \pm .351$ , standard deviation 3.25. The Jersey cow which falls in the 100 centimeter class should probably be eliminated since she is a nymphomaniac. (Four other nymphomaniacs were included in the Jersey group. All four were at the upper limit of the range in round measurement as well as body weight.) The round measurements of the

TABLE 4  
*Round measurement in centimeters of mature cows of all breeds.*

BREED	84 83-85	87 86-88	90 89-91	93 92-94	96 95-97	99 98-100	102 101- 103	105 104- 106	108 107- 109	111 110- 112	114 113- 115	TOTAL	MEAN	S.D.
Jersey	5	11	8	7	4	1						39	90.75 ± .351	3.25
Guernsey	1	1		1										
Ayrshire			1		1	1	2	1	1			7	100.92 ± 1.41	5.55
Holstein						1	3	5				9	103.94 ± .472	2.10
Shorthorn						1	3	4	3	2		13	106.11 ± .657	3.52
Hereford						2		4	2	1	1	13	106.75 ± .755	4.04
Angus								1	2					

Shorthorns range from 99 to 112 centimeters with a mean of  $106.11 \pm .657$ , standard deviation 3.52. The Hereford-Angus group ranges from 100 to 115 centimeters with a mean of  $106.75 \pm .755$ , standard deviation 4.04. The number of Holsteins is slightly more limited than some of the other groups, the range being from 99 to 106 centimeters, with a mean of 103.94.

When compared with the Jersey-Guernsey group on the one hand, and the beef breeds on the other, the Ayrshires present some very interesting data. It should be recalled that this group is near the same height as the other breeds (Holsteins excepted) but is intermediate in round measurement, the range being from 90 to 107 centimeters. The Ayrshire is a dairy breed, but some strains have been selected along dual purpose lines. Some consideration should be given to the number of different genetic capacities for round measurement. When the mean round measurement of the Jersey-Guernsey group is compared with that of the Holsteins the difference is  $13.19 \pm .588$ . This difference is 22 times the probable error. The comparison of round measurement of the Jersey-Guernsey group is not made with either of the beef groups since it is clearly evident that the difference is significant without this analysis. When the mean round measurement of the Holsteins is compared with that of the Hereford-Angus group the difference is  $2.81 \pm .915$ . This difference is only three times the probable error and is of doubtful significance.

The next question for consideration is how many different genetic complexes are there for round measurement or the development of muscle diameter? There are unmistakably two, and possibly more—one for the Jersey-Guernsey group and one for the beef breeds (Hereford, Angus and Shorthorns). In round measurement the Holsteins are somewhat intermediate between the Jersey-Guernsey group on the one hand and the beef breeds on the other, but on account of their great discontinuity for height it is difficult to interpret and evaluate the significance of their round measurement. The Ayrshire breed indicates that some segregation is oc-

curring so far as muscle diameter is concerned. This is indicated by the variability of the round measurement. In muscle development they are intermediate between beef and dairy breeds and tend to range over the whole series. This appears to agree with the observations of KUHLMAN (1915) in crosses between Jersey and Angus cattle.  $F_1$  hybrids of this cross were somewhat intermediate, but were nearer the Angus or beef type of conformation, while the  $F_2$  and subsequent generations showed greater variability in types of conformation. It is unfortunate that a larger number of the Ayrshires was not available for analysis.

#### WEIGHT IN MATURE ANIMALS

On account of numerous environmental factors which one cannot control, it is exceedingly difficult to standardize weight in mature cows. Most of this variability is caused by change in condition such as varying amounts of fat, pregnancy and, in dairy cows, lactation.

In their investigations at the Missouri station, TROWBRIDGE, MOULTON and HAIGH (1918, 1921, 1922, 1923) showed that in Hereford-Short-horn cattle, the greatest increase in fatty tissue takes place in the plate, loin and flank. An increase also takes place in the rib and rump, but to a slightly less degree. Now, since only a slight amount of fat is deposited in the region of the round, appreciable changes in body weight caused by variation in amount of body fat, can take place without causing a corresponding change in the round. This fact is demonstrated by Hereford cow number 199. On September 16, 1930, her round measurement was 105 centimeters and her weight was 1290 pounds. On February 24, 1931, after an illness, caused by a uterine infection, she had lost 315 pounds, but retained a round measurement of 104 centimeters—a loss of only 1 centimeter. In other words, she suffered a considerable change in body weight which was unquestionably due to loss of fat, but her round measurement remained within the limit of the error of measurement. This instance gives additional evidence that the round measurement is primarily a measurement of muscle diameter.

In table 5 are summarized the weights of all the mature cows shown for height in table 3 and round measurement in table 4. The Jersey-Guernsey group ranges from the class value of 924 to 1324 pounds with a mean of  $1076 \pm 11$ , standard deviation 103. The Hereford-Angus group ranges from 1074 to 1574 pounds with a mean of  $1334 \pm 27$ , and a standard deviation of 146. The Shorthorns range from 1174 to 1724 pounds. They have a mean of  $1432 \pm 26$ , and a standard deviation of 139. The Holsteins range from 1224 to 1524 pounds with a mean of 1375. In this analysis of weight, the Ayrshires tend to be intermediate between the Jersey-Guernsey group and the beef group.

TABLE 5  
*Weight in pounds of mature cows of all breeds.*

BREED	924- 974	1024- 1074	1124- 1174	1224- 1274	1324- 1374	1424- 1474	1524- 1574	1724- 1824	TOTAL	MEAN	S.D.
Jersey	10	11	11	3	1				39	1076.3 ± 11.1	103.16
Guernsey	1	1	1								
Ayrshire	1		1	3	1	1			7	1239.2 ± 35.8	140.69
Hereford		1	1	2		5	1		13	1334. ± 27.3	146.15
Angus			1			2					
Holstein				2	4	2	1		9	1375. ± 19.4	86.6
Shorthorn			1	1	3	4	3	1	13	1432.6 ± 26.1	139.8

RELATION OF HEIGHT, MUSCLE DEVELOPMENT AND WEIGHT  
IN MATURE ANIMALS OF DIFFERENT BREEDS

CASTLE (1914 and 1922) found in the rabbit that for a given linear skeletal length there is a proportional amount of development of other tissues. Evidently, linear skeletal measurements and muscle diameter are

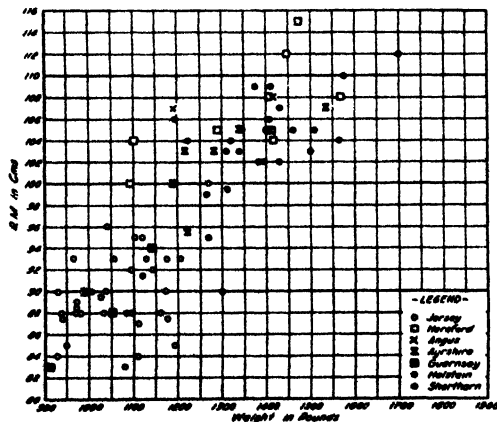


FIGURE 2.—Round measurement plotted against weight for mature cows of all breeds.

highly correlated with each other and both are correlated to about the same degree with total body weight. Now, if the same condition prevails in cattle these correlation coefficients should also prevail. The correlation coefficients have been determined for the mature cows shown in tables 3, 4 and 5 and the results, which are exceedingly interesting, are shown in table 6. Since the Holsteins are discontinuous with all the other breeds so far as linear skeletal development is concerned, correlation coefficients were determined both with and without the Holsteins.

When all breeds are considered together, the correlation coefficient of round measurement, the criterion of muscle development, with weight is

$+ .875 \pm .017$ . When the Holsteins are omitted only the breeds which are generally similar in linear skeletal development remain. The correlation coefficient of round measurement with weight is  $+ .896 \pm .015$ . This is slightly higher than when the Holsteins are included. It is clearly evident from this that muscle development is highly correlated with body weight.

When all breeds are considered together, the correlation coefficient of height, the criterion of linear skeletal development, with weight is  $+ .383 \pm .064$ . If the Holsteins are omitted the correlation coefficient is  $+ .040 \pm .079$ . The evidence indicates that in mature cows linear skeletal development with associated muscle lengths is not the most important factor affecting weight. Furthermore, it indicates that some of the genetic

TABLE 6  
*Correlation coefficients of mature cows.*

	TOTAL	CORRELATION COEFFICIENT	
R. M. with weight (all breeds)	80	$+ .875$	$\pm .017$
R. M. with weight (Holsteins omitted)	71	$+ .896$	$\pm .015$
R. M. with weight (Mature cows all of the same height class of 125-127 centimeters)	30	$+ .889$	$\pm .021$
Height with weight (all breeds)	80	$+ .383$	$\pm .064$
Height with weight (Holsteins omitted)	71	$+ .040$	$\pm .079$
R. M. with height (all breeds)	80	$+ .190$	$\pm .072$
R. M. with height (Holsteins omitted)	71	$- .016$	$\pm .08$

agencies which control muscle diameter differ from and are possibly independent of (not linked with) those which control linear skeletal development. A more critical test of this is made when round measurement (the

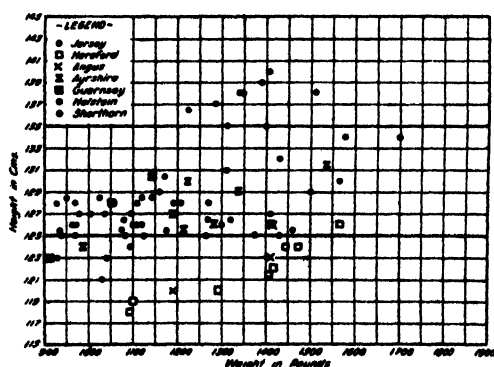


FIGURE 3.—Height plotted against weight for mature cows of all breeds.

criterion of muscle development) is correlated with height at withers, the index of linear skeletal development. When the round measurements of mature cows of all breeds are correlated with their heights the correlation coefficient is  $+ .190 \pm .072$ . When the Holsteins are omitted this correlation

coefficient drops to  $-.016 \pm .08$ . A still more critical test to determine the dependence or independence of the genetic agencies which affect muscle diameter and linear skeletal development was made by eliminating the effect of linear skeletal development. This was accomplished by taking all mature animals in the height at withers class of 125 to 127 centimeters and correlating their round measurements with their weights. Obviously all Holsteins were omitted. The correlation coefficient for the thirty animals tested in this manner was found to be  $+.899 \pm .021$ , which is almost as high as  $+.896 \pm .015$  the correlation coefficient of round measurement with weight when the Holsteins were omitted.

The evidence obtained from the analysis by means of correlations appears to be conclusive proof that some of the genetic agencies which control the development of muscle diameter in mature animals differ from and are possibly, though not necessarily, genetically independent of those which control linear skeletal development.

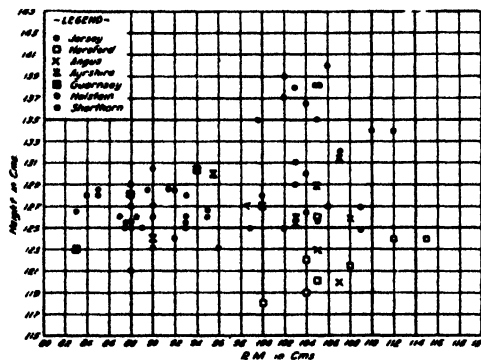


FIGURE 4.—Height plotted against round measurement for mature cows of all breeds.

The data concerning the immature animals as a whole are numerous, but when restricted to narrow limited age groups, they are not numerous enough to analyze for any particular age class when the relations of muscle development and total body weight are considered. For this reason another method of analysis is used for determining the nature of growth factors in animals of all ages.

#### RELATION OF HEIGHT, MUSCLE DEVELOPMENT AND FAT TO WEIGHT IN NEWLY-BORN CALVES OF DAIRY AND BEEF BREEDS

Some consideration should be given to the development of muscle, skeleton, and fat in relation to total body weight in newly-born calves of different breeds. Although we have no large mass of data bearing on this subject, what we have is sufficient to establish certain important facts. The calves included in this analysis range from one day to sixteen days of age.

The majority, however, is seven days or less. The age class range should have narrower limits. The data are summarized in table 7.

TABLE 7  
*Height, weight, and round measurement in newly-born calves of different breeds.*

			AGE IN DAYS	HEIGHT	ROUND MEASUREMENT	WEIGHT
Jersey	Female	512	13	65.5	39	52
	"	513	4	64.5	37.5	45
	"	514	5	65.5	40	47
	Male	58E	9	61.0	44	55
	"	57E	10	68.0	44	63
	Female	520	4	70	37	53
	Male	(Dam 426)	3	63.5	40.5	52
	"	(Dam 402)	2	68.5	41.0	63
	"	(Dam 444)	2	69.0	40.0	52
	Female	521	1	58	33.5	34
Guernsey	"	522	1	65.5	41	54
	Male	607A	1	69	44.5	64
Mean				65.6	40.1	52.8
Hereford	Female	436	8	67.5	55	100
	"	437	7	70	58	
	"	448	7	65.5	46	67
	"	449	4	57.5	39.5	45
	"	450	16	65.0	53	82
Angus	"	434	7	65.0	53	90
Hereford	"	446	6	65.0	51.0	75
Angus	"	432	16	65.5	54.0	100
	"	433	16	68.0	55.0	115
	"	482		64.5	42	48
Shorthorn	"	445	11	65.0	47	75
Twins	{ Male	463	14	65.0	45	75
	{ Male	464	14	66	47.5	80
Mean				65.3	49.7	79.3
Holstein	Female	183	9	72	51	90
	"	184	3	71	50.5	86
	"	187	2	68	45	61
	"	188	5	72	52	84
	"	189	1	71.5	46.5	81
Mean				70.9	49	80.4
Ayrshire	Female	249	7	71.5	53	82
	"	250	7	69	50.5	85

The range in height of the Jersey-Guernsey group is from 58 to 70 centimeters with a mean of 65.6. The range in round measurement is from

33.5 to 44.5 centimeters with a mean of 40.1. The weight ranges from 34 to 64 pounds with a mean of 52.8.

The range in height for the Hereford-Angus-Shorthorn group is from 57.5 to 68 centimeters with a mean of 65.3. The round measurement varies from 39.5 to 58 centimeters with a mean of 49.7. The range in weight is from 45 to 115 pounds with a mean of 79.3. Since the number of Holsteins and Ayrshires is limited, no attempt has been made to analyze the results. However, the weight of the two Ayrshire calves is slightly higher than the mean birth weight of 72 pounds as given by ECKLES. The Holstein calves appear on the other hand, to weigh slightly less than the ones reported by him. The mean weight of the Jerseys, 52.8 pounds, closely approximates the mean birth weight of Jerseys reported by ECKLES (1919) for the Missouri herd.

The mean weight of the newly-born Hereford-Angus-Shorthorn calves, is 79.3 which is close to that given by HAIGH, MOULTON and TROWBRIDGE (1920). They reported that four calves from dams which were on a high plane of nutrition averaged 82.53 pounds, while five calves from dams on a medium plane of nutrition averaged 80.87 pounds.

When the height of the Jersey-Guernsey group is compared with that of the beef group, it slightly exceeds the latter, though the difference is insignificant. On the other hand, when the mean round measurement and weight of the Jersey-Guernsey group are compared with those of the beef group, the beef group unmistakably exceeds in both of these measurements. Here again we find that weight is related to round measurement (muscle diameter).

Is the difference in weight between the newly-born Jersey-Guernsey calves on one hand, and the beef calves on the other, caused by differences in muscle development or in the amount of fat? The investigations of HAIGH, MOULTON and TROWBRIDGE (1920) on the composition of the bovine at birth, give some information concerning this question. Jersey calves 11-A and 11-B contained 4.591 and 3.494 percent of fat respectively in the total carcass. Four Hereford calves on a high plane of nutrition contained from 3.8 to 4.4 percent fat with a mean of 4.155 in the entire carcass. Five Hereford calves on a medium plane of nutrition had a range of 3.2 to 3.95 percent of fat in the entire carcass. The mean was 3.568. From the study at the Missouri station, it is evident that differences in total fat of the newly-born calves of dairy and beef breeds will not account for differences in birth weight, but differences in development of muscle diameter will account for a large part, if not all, of the difference in birth weights.



## GENERAL CONFORMATION

*a. Mature cows*

Since ECKLES, and ECKLES and SWETT, showed that almost any linear skeletal measurement may be calculated with a high degree of accuracy from the measurement of the height at withers alone, it is evident that in cattle the factors which control linear skeletal development are chiefly, if not exclusively, general in their effect. This also seems to agree with WRIGHT's analysis for skeletal development for rabbits and White Leghorn fowls. The question then arises as to whether or not the agencies which control the development of muscle diameter are chiefly general with group and specific factors having a minor effect. WRIGHT's methods of analysis should definitely settle this question. However, since it was not practical to make such an analysis, a more indirect method was used.

In the mature animals, the correlation coefficients of round measurement with weight was very high as shown in the previous discussion and in table 6. The three correlation coefficients of round measurement with weight were  $+.875, \pm .017$ ;  $+.896, \pm .015$ ; and  $+.889 \pm .021$ , depending upon the grouping of the animals used in the study. From these it is evident that some of the agencies which control the development of muscle diameter have a general effect and indicate that many muscles were influenced. Furthermore, if specific factors which affect individual muscles or groups of muscles are involved, they evidently play a subordinate role in their total effect on body weight. For this reason, from the standpoint of expressing the build or general conformation (relation of muscle diameter to linear skeletal development) of an animal one may disregard the possible effect of group or specific growth factors on the development of muscle diameter as well as on linear skeletal development, though it is highly probable that some are involved in both systems.

By taking the round measurement as the numerator and the linear skeletal measurement of height at withers as the denominator ( $R.M./H$ ) one may express general conformation in statistical units. This expression gives an abstract number which represents in a general way the ratio of muscle diameter to linear skeletal development, and which is diagnostic of build or general conformation. This standard of the measurement of conformation is referred to in this paper as the muscle-skeletal index or ratio. Table 8 shows the summary of the muscle-skeletal index of mature cows of all breeds studied. Since all of these numbers are decimals, it may be more convenient to express the index as whole numbers by omitting the decimal point, but in this analysis the decimal point is retained though in table 8 the decimal point is omitted and the index is carried to two figures only.

TABLE 8  
*Index of mature cows of all breeds.*

BREED	GROUP NO.	RANGE IN INDEX	TOTAL	MEAN	S.D.	GROUPS COMPARED	MEAN DIFFERENCES	DIF. P. E.
Jersey } Guernsey }	1	65-77 } 66-72 }	39	71 ± .3	3.1	1-2	4.4 ± .46	9.5
Holstein	2	72-78	9	75 ± .3	1.4	2-4	6.9 ± .55	12.4
Ayrshire	3	72-82	7	79 ± .9	3.7			
Shorthorn	4	78-87	13	82 ± .4	2.4	4-5	5.0 ± .65	7.7
Hereford } Angus }	5	82-93 } 84-89 }	13	87 ± .5	2.5			

The Jersey-Guernsey group has a range in the muscle-skeletal index from .6595 to .7795 with a mean of  $.709 \pm .003$ , and standard deviation of .031. The Holsteins range from .7295 to .7795 with a mean of  $.753 \pm .003$ , standard deviation .014. Although limited in number, the Ayrshires show two trends of type of conformation, one a dairy type and the other beef. In spite of this, however, the Ayrshires may be classed as somewhat intermediate between the beef and dairy types of conformation. They range from .7295 to .8195 with a mean of  $.787 \pm .009$ , standard deviation .037. The muscle-skeleton index of the Shorthorns ranges from .7895 to .8695 with a mean of  $.822 \pm .004$ , standard deviation .024. The Hereford-Angus group ranges from .8295 to .9295 with a mean of  $.872 \pm .004$ , standard deviation .025.

Since Holsteins are discontinuous with the other breeds for skeletal development, and since the Ayrshires are somewhat intermediate in conformation between the beef and dairy types, we shall compare the Jerseys and Guernseys with the combined beef breeds, Shorthorn, Hereford, and Angus. The mean muscle-skeletal index for the Jersey-Guernsey group is  $.709 \pm .003$ , while it is  $.847 \pm .001$  for the combined beef breeds. When the probable error of the difference is considered, we find that the deviation is more than thirty-eight times the probable error of the difference.

From this analysis it is evident that mature cows of the various breeds, or even individual animals which differ in conformation (the ratio of muscle to skeleton) may be separated by the muscle-skeleton index. Expressing the difference in conformation in statistical units on the basis of the muscle-skeletal index is a new method of attack and it may aid in the solution of the complex problem of the inheritance of conformation and size.

*b. The muscle-skeleton index in different breeds of all ages*

Evidence has been presented which indicates that for mature animals, the muscle-skeleton index is diagnostic of differences in conformation be-

tween the various breeds of cattle, and even between individuals of the same breed when they happen to differ in build. Now the question arises as to whether or not differences in conformation exist throughout the growing periods from birth to maturity. An appreciable amount of data has been gathered which bears on this question. Individuals of all breeds and of all ages from birth to maturity may be used in this analysis if the muscle-skeleton index is plotted against age in months. These data are given in the form of a graph and shown in figure 5. This chart clearly shows several striking facts. The method of expressing conformation in statistical units shows a distinct difference in conformation between beef and dairy breeds from birth to maturity. The figure .785 approximately separates the beef from the strictly dairy breeds. In the foregoing discussion it was stated that so far as dairy and beef types are concerned, the Ayrshire

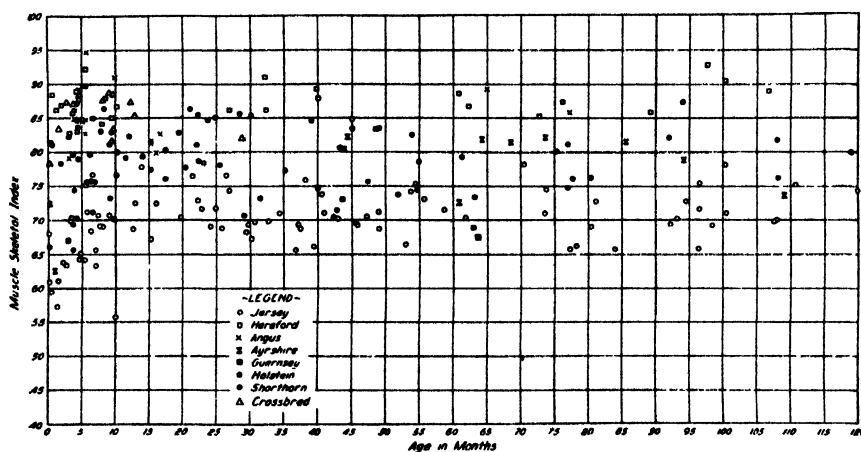


FIGURE 5.—The muscle-skeletal index of animals of all breeds and all ages plotted against age in months.

cows in the Experiment Station herds are of mixed types of conformation. When we examine the analysis in figure 5 this fact is clearly shown. All of the mature Holstein cows fall below the beef breeds in muscle-skeleton index except a few young Holsteins from four to twenty-six months of age, which plot in the lower limit of the range of the beef group.

When the beef breeds are considered together, the Hereford and Angus breeds tend to fall together, and have a muscle-skeleton index slightly higher than the Shorthorns. The beef breeds at birth have a muscle-skeleton index which they tend to retain throughout the growing and adult periods.

The Jerseys and Guernseys plot together and have a low muscle-skeleton index. The Holsteins have a muscle-skeleton index which is slightly higher than that of the Jersey-Guernsey group. The dairy breeds give us a

very interesting picture of conformation from birth to maturity. At birth the ratio of muscle diameter to linear skeletal development for the Jerseys is about .60. This is low. However, the index gradually changes and by the time the Jerseys are eight months of age the muscle-skeletal index has become relatively constant and remains so throughout the growing and adult period. There is an indication that a change in muscle-skeleton index takes place likewise in the Holstein breed, but it is less marked. In other words, the muscle-skeleton index for the Jerseys tends to change from birth



FIGURE 6.—Photograph of mature Jersey cow 421. Height 126 centimeters, round measurement 88.5 centimeters, weight 970 pounds. Note the lack of development of muscle in the round and throughout the body. Muscle-skeletal index 702. Compare with figure 7.

to about eight months of age, whereas that of the beef breeds tends to remain constant from birth to maturity.

A question which deserves consideration concerns the genetic agencies which operate to affect conformation so far as muscle diameter and linear skeletal development are involved. These factors, which affect muscle diameter as found in the breeds studied, are evidently different in nature and are possibly for the most part genetically independent of those affecting linear skeletal development.

There should be as many types of conformation in cattle as it is possible to obtain by uniting all genotypes affecting muscle diameter with all genotypes affecting linear skeletal development.

*Relation of the muscle-skeletal ratio to pre-natal  
and post-natal development*

Since the beef breeds start at birth with a muscle-skeletal ratio which remains practically constant throughout life, and since the dairy breeds, especially the Jersey, have a low muscle-skeleton index at birth, but have an increase in amount of muscle in relation to their linear skeletal development for the first eight months, at which time it becomes practically constant and remains so throughout the remainder of the life of the individual, it is clearly evident that the embryological and foetal develop-

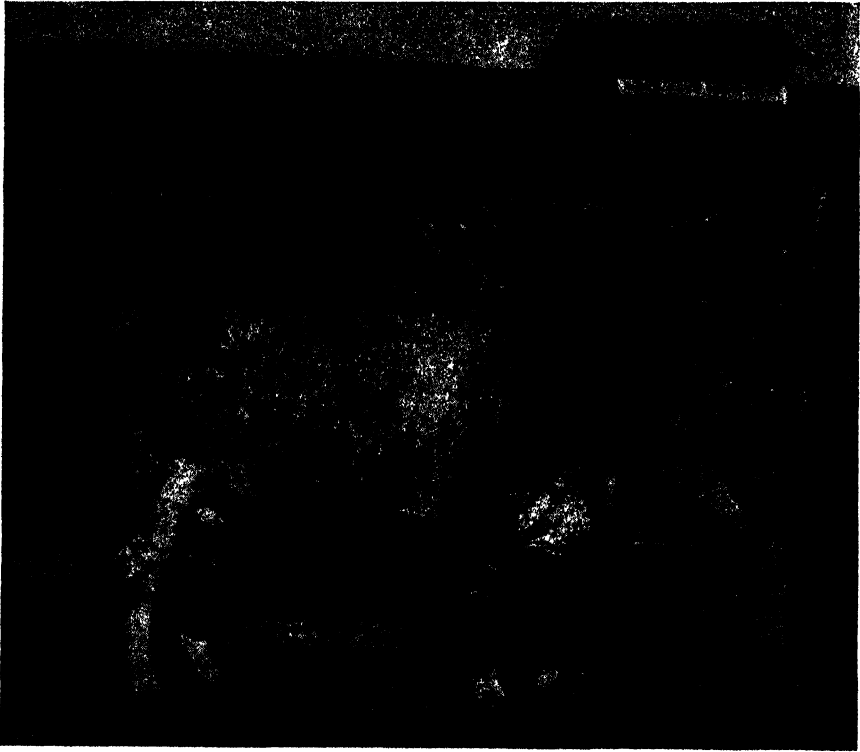


FIGURE 7.—Photograph of mature Shorthorn cow 212. Height 126.5 centimeters, round measurement 104 centimeters, weight 1320 pounds. Note the excessive development of muscle in the round and throughout the body. Muscle-skeletal index 822. Compare with figure 6.

ment of the beef breeds differ in some detail from that of the Jersey breeds. At birth the beef breeds appear to have their adult muscle-skeleton ratio, while it takes the Jerseys several months of post-natal development to reach their adult muscle-skeleton ratio. This seems to indicate that the stimulus which causes the development of muscle tissue is less marked in some dairy breeds during the foetal period than in the beef breeds. This seems to agree in part at least with the condition reported in the rabbit

by CASTLE and GREGORY (1929) and GREGORY and CASTLE (1931). They found that the eggs of large races of rabbits segment at a faster rate than those of small races. Now, since some of the genetic agencies which control the development of muscle diameter in cattle are different from and exhibit a certain degree of independence from those which control linear skeletal development, it appears logical to assume that the biochemical substance which causes muscle development is more concentrated in the beef breeds during foetal development than it is in the dairy breeds.



FIGURE 8.—Posterior view of Jersey cow 421. Note the deep depression and lack of muscle development in the round. Compare with figure 9.

GREGORY and GOSS showed that in the rabbit the concentration of sulphhydryl is greater in large race rabbits than in small race rabbits. The chemical determinations were based on the analyses of newly-born rabbits which had fasted 48 hours. At this time it is impossible to state the biochemical nature of the substance which stimulates muscle development in cattle, but it may be the concentration of sulphhydryl since this biochemical compound is known to stimulate growth by increase in cell number as was shown by HAMMETT and his associates.

#### DISCUSSION

##### *The nature of growth factors in mammals*

The evidence from the data presented in this paper indicates that some of the genetic agencies which control linear skeletal development in domes-

tic breeds of cattle are different from and possibly independent of those which control muscle volume. From an analysis of the data of MACDOWELL and CASTLE (1914), CASTLE (1922) for three populations of rabbits, and from DUNN's data on the White Leghorn fowl, WRIGHT (1932) seems to have definitely established the existence of three types of growth factors—general, group and specific—which influence skeletal development and weight. General growth factors (with an exception in the  $F_1$  rabbit population) account for most of the total effect, while group and specific factors each contribute a minor part. WRIGHT's interpretation of growth factors

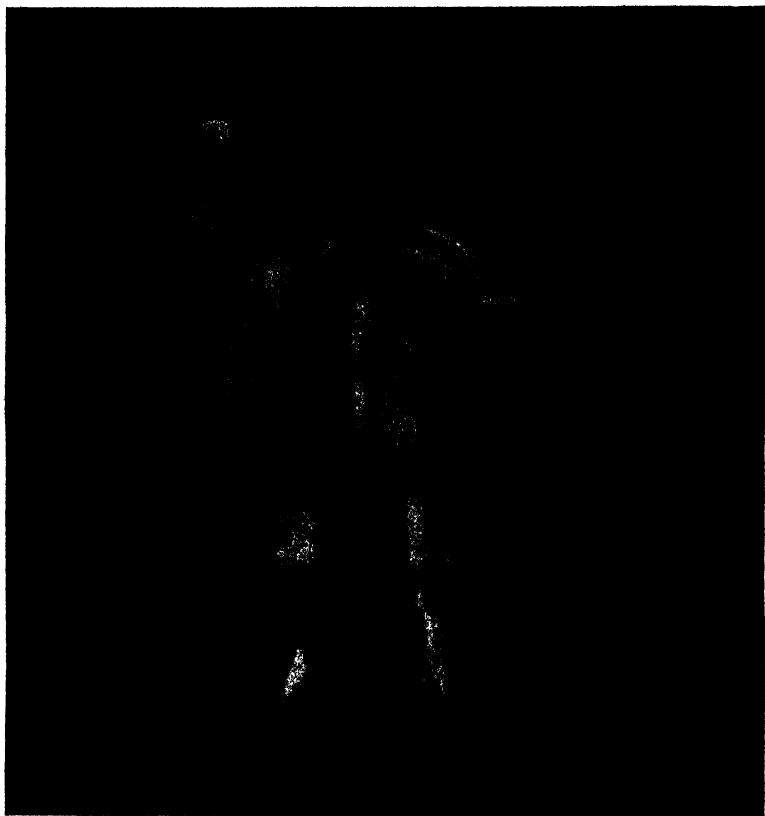


FIGURE 9.—Posterior view of Shorthorn cow 212. Note the lack of depressions and full muscle development of the round. Compare with figure 8.

is in harmony with the observations and conclusions of SUMNER and GREEN for mice.

In several recent publications GREEN gave the results of his work on size inheritance in a mouse species cross between *Mus musculus* and *Mus bactrianus*. In his report on the nature of size factors, he gave the correlation coefficients of various skeletal measurements, most of which were linear, in relation to each other and in relation to total body weight.

This was done for the two species mentioned above, as well as for the  $F_1$  hybrids between the two. From his analysis GREEN came to the conclusion that general and specific factors are both involved in development. The data presented by GREEN seem to indicate that some of the genetic agencies which control linear skeletal development may be independent of some of the genetic agencies which control the development of weight. If this is the case it agrees with the findings in cattle.

In the mouse species cross, GREEN had three chromosomes marked with genes A, B and D. The *musculus* parent carried the recessive genes while the *bactrianus* parent carried their dominant allelomorphs. Since all the brown mice which were produced from  $F_1$  hybrids back-crossed to the *musculus* parent were heavier and had greater bone dimensions (with the exception of skull length and width) than the black mice produced from the same matings, GREEN came to the conclusion that the *musculus* chromosome bearing the brown gene also bore a factor for increased linear skeletal measurements.

GREEN's analysis does not determine whether or not this growth factor which is borne in the chromosome which bears the brown gene affects other tissues in the body, such as muscle diameter.

Since conformation, the relation of muscle diameter to linear skeletal development, is an important factor in weight and size inheritance, and since differences in conformation seem never to have been taken into account in genetic studies of growth and size inheritance, development of muscle diameter and its effect on weight may be one of the disturbing factors in the interpretation of the nature of growth factors. Since general, group and specific factors influence linear skeletal development, these three types of growth factors may also be involved in influencing muscle diameter and, consequently, volume.

The evidence presented in this study indicates that general factors are responsible for most of the growth in each system (muscle and skeletal), but in neither system does it evaluate their total effect or the influence of group and specific factors. Furthermore, it would be desirable to know if growth factors exist which operate on the development of both muscle diameter and the linear skeleton. In the preceding pages it was shown that Holsteins are discontinuously greater for height at withers (linear skeletal development) and at the same time have an increased round measurement. Can this increased linear skeletal development and increased round measurement be accounted for by a general factor (or factors) which affects both muscle diameter and linear skeletal development, or is size in Holsteins accounted for by factors which increase linear skeletal development combined with factors which cause greater muscle diameter? This study does not answer this fundamental question.



So far as the relation of muscle diameter to linear skeletal development in the beef and dairy animals used in this study is concerned, there are several unmistakably contrasting types of conformation. This in itself is evidence that some of the growth factors which affect development of muscle diameter are different in nature and possibly, though not necessarily independent of (not linked with) those which affect linear skeletal development.

We believe that the muscle-skeleton index may be of value in the analysis of some of the problems of conformation and size inheritance in cattle, and possibly other animals, since it enables one to express general conformation in statistical units. If the factors which influence muscle diameter and linear skeletal development were exclusively general in each system this index, fundamentally at least, should be an almost perfect criterion of conformation. However, it is a practical certainty that group and specific factors are operating to limited extents in both the muscle and the skeletal systems. Therefore, this expression of conformation (relation of muscle diameter to linear skeletal development) is without doubt subject to slight errors which are caused by group and special factors operating in each system. When we determine the exact role that general, group and specific factors play in the development of both muscle diameter and the linear-skeleton, then the absolute value of the muscle-skeleton index may be determined. Whatever the outcome of that study may be, I believe that the preponderance of general growth factors operating in both the muscle and skeletal systems will cause the muscle-skeletal index to have some value as a tool to aid in the analysis of the inheritance of conformation and related phenomena in cattle.

GREEN has shown for mice that the male sex hormone affects the expression of growth factors. He suggested that growth factors are more specialized in males and more general in females. WRIGHT has shown that sex has only a slight effect on growth in rabbits. Since no mature males are used in this study of cattle, we are not certain of the effect of the male sex hormone on conformation. It is evident that the male sex hormone causes a certain amount of modification of conformation in cattle. The magnitude of this modification is yet to be determined.

#### SUMMARY

1. A study of the nature of growth factors which control muscle diameter and linear skeletal development was made in Hereford, Angus, Shorthorn, Holstein, Jersey, Guernsey and Ayrshire cattle. Most of the animals used were females, but a few males were used in the younger ages.
2. From an analysis by means of correlation coefficients, evidence is presented which indicates that some of the genetic agencies which control

muscle diameter are different in nature and possibly, though not necessarily, genetically independent of the genetic agencies which control linear skeletal development.

3. Evidence is also presented which indicates that some of the factors which control the development of muscle diameter are chiefly general in nature. However, the data do not deny the existence of group and specific factors influencing the development of muscle diameter.

4. There are at least two different genetic compositions which are clearly demonstrated, involved in the development of muscle diameter. It is yet to be demonstrated whether or not other genetic compositions having marked effects are involved.

5. From the study of ECKLES and SWETT on linear skeletal development in cattle the conclusion is reached that the genetic agencies affecting linear skeletal development are chiefly general in nature and if specific factors affecting individual bones, or groups of bones, are involved they play only a minor part in linear skeletal development.

6. The evidence indicates that in the breeds studied, at least three different genetic compositions are involved in height, the criterion of linear skeletal development, and there is some indication of a fourth.

7. Since linear skeletal development in cattle is controlled by growth factors which are chiefly general in nature, and muscle diameter is controlled by growth factors which are also chiefly general in nature, and since genetic agencies which control linear skeletal development are different from and possibly independent of the genetic agencies which control muscular diameter, the general conformation of an animal may be expressed in statistical units by taking the round measurement as the numerator and the height as the denominator  $\left(\frac{R. M.}{H}\right)$ . This represents in a general way the ratio of muscle diameter to linear skeletal development. The conclusion is reached that conformation is primarily the result of the interaction of growth factors (general group and specific) affecting muscle diameter combined with growth factors (general, group and specific) affecting linear skeletal development.

8. Evidence from the study of the muscle-skeleton index also indicates that the genetic agencies which affect the development of muscle diameter have a certain degree of independence from those which affect linear skeletal development.

9. The muscle-skeletal index of the beef breeds studied was practically constant from birth to maturity. In the dairy breeds, especially Jerseys, the muscle skeleton index was not constant from birth to maturity. The evidence indicates that in Jerseys, muscle tissue is retarded during foetal development and the adult muscle-skeletal index is not reached until the

young are about eight months of age. This indicates that muscle development in beef breeds is somewhat more accelerated than in dairy breeds (with the exception of Holsteins) during foetal development, while the rate of linear skeletal development of beef and dairy breeds is about the same.

10. When the nature of growth factors, as reported in mice and rabbits, is considered in relation to the interpretation made for different breeds of cattle, the conclusion is reached that differences in conformation in the races or species studied may account for some of the diversity of opinions held on the general or specific nature of growth factors.

#### ACKNOWLEDGMENTS

The author wishes to express his indebtedness and appreciation to several of his colleagues and others who have aided in this investigation. Without the encouragement of Professor HART, Chairman of the Division, this investigation would have not been possible. I am indebted to Mr. GUILBERT for the use of the beef herd and for aid in making some of the measurements, also to Mr. ALEX MACDONALD, herdsman at the beef barn, for aid and cooperation in taking all the measurements of the beef herd. To Professor REGAN, Mr. MEAD and Mr. FOLGER, who have charge of the dairy herd, I am indebted for the use of the dairy animals and their whole-hearted cooperation throughout the study, and also their aid in making some of the measurements of the dairy cattle. Mr. STOTTEMEYER, herdsman at the dairy barn, also gave valuable cooperation and assistance. Mr. KENNETH WAGNON, a graduate student, assisted in making many of the measurements and in the analysis of some of the data.

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# A CLOSED X CHROMOSOME IN *DROSOPHILA MELANOGASTER*\*

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From a pair of attached X chromosomes of *Drosophila melanogaster* there is derived occasionally a single detached X chromosome which shows all the characteristics of a normal X. In consequence a very small class of regular offspring is found in attached X lines. One yellow female having attached X chromosomes mated to scute broad apricot males produced a regular wild-type female, the phenotype to be expected if an egg bearing a detached X had been fertilized by X sperm; but among the offspring of the wild-type female there was an unexpected class of males. She had been mated to a forked bar male and produced, besides about 12 yellow and 35 scute broad apricot males, 7 forked bar males. The wild-type female had received one yellow-bearing chromosome from her mother and an X chromosome bearing genes for scute broad apricot from her father. If she had received in addition to a single yellow-bearing chromosome also a Y chromosome from her attached X mother, exceptional males would have been expected from Y eggs, but secondary exceptional wild-type females would also have been expected. In fact all of the daughters were heterozygous bar; moreover all of the forked bar males were found to be sterile, and were evidently primary non-disjunctional XO males. Some of the F<sub>1</sub> heterozygous bar females were mated to X-ple males (*sc ec cv ct<sup>6</sup> v g<sup>2</sup> f*) and again sterile non-disjunctional males were produced, and among the regular classes there were unusual ratios (table 1). Whereas about 50 percent of crossing over is expected between yellow and forked, only 6 among 225 regular males showed recombination of the characters.

TABLE 1

Offspring of $\frac{X^{ey}}{fB} \text{ } \varnothing \times \text{X-ple } \sigma^1$ .							
NO. OF CULTURE	$\varnothing \varnothing$		$\sigma^1 \sigma^1$				
			NON-CROSSOVERS		CROSSOVERS	PATOCLOINOUS	
	+	fB	y	fB	y/fB	+	X-ple
2.54a	70	82	56	87	0	3	2
2.54b		166	48	58	0	3	1

\* Part of the cost of the accompanying tables is paid from the GALTON AND MENDEL MEMORIAL FUND.

The yellow-bearing chromosome for two generations had shown certain peculiarities which were hereditary. Further tests showed that the chromosome is unique, in that among the offspring of females homozygous or heterozygous for the chromosome there are no single or triple cross-overs, but there are many patroclinous males and gynandromorphs are unusually frequent (L. V. MORGAN 1929).

## CYTOLOGY

The unique chromosome in preparations of oögonial plates appears to be a thick almost circular ring, clearly hollow in the middle. The ring

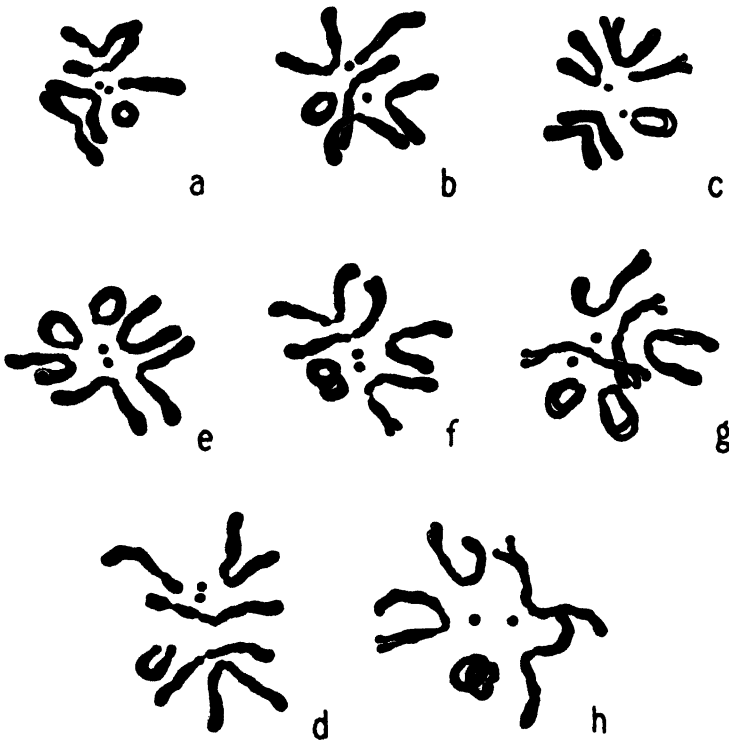


FIGURE 1.—The drawings represent metaphase-plates from the nerve cells of the ganglia of larvae of females carrying closed  $X^0$  chromosomes. Figures a-d (from heterozygous females) contain one closed  $X^0$  chromosome. Figures e-h (from homozygous females) contain two closed  $X^0$  chromosomes. The drawings were made with the aid of camera lucida, at the level of the work table. The magnification is about 5300.

often appears to be closed, but at some optical levels it is sometimes slightly open on the side toward the centre of the plate, which led to its description "an almost or entirely closed and somewhat rounded letter U" (L. V. MORGAN 1926). Dr. DOBZHANSKY has very kindly made preparations and drawings of metaphase plates from the nerve-cells of the ganglia of female larvae (figure 1); in these the chromosomes are less contracted and

details of their structure are more clearly seen. In the larval nerve-cells it appears that the two ends of the curved chromosome are indeed in contact (as seen in all figures except one, figure 1d), and that they are directed toward the centre of the plate. It appears also that the ends of the chromosome are thinner than the middle, hence the apparent opening in the more contracted oogonial chromosomes, especially if the chromosome is inclined to the plane of the section, when at some optical levels only the thicker sides of the chromosome are seen.

When the chromosome lies flat in the plane of the section it appears like a ring sometimes elongated in the axis which passes between the ends. It can be interpreted as an X chromosome of which the spindle fibre end is as usual in a position near the centre of the metaphase plate and in which the usually free end of the chromosome has been brought into contact with the spindle fibre end. Thus the cytology presents an X chromosome whose ends are united to each other forming a ring or oval such that interchange between two chromatids can form a single chromatid only when exchange has taken place at two points simultaneously.

Dr. DOBZHANSKY's figures show further that, when the chromosome divides, the split seems not to begin at the end, as occurs when the end is free, but in the middle of the closed chromosome (figure 1c and 1g).

The genetic and cytological results can be explained on the hypothesis that the unique X chromosome is a closed chromosome in which the left end is attached to its own right end near the spindle fibre attachment.

#### ATTACHMENT OF THE ENDS OF THE CLOSED CHROMOSOME

There is evidence from genetic tests that the point at which the ends are united is close to genes at the extreme left end and at the right end of the chromosome.

First, there is abundant evidence that the left end of the closed chromosome which carries the gene for yellow ( $y$ ) is never separated by crossing over from the right end carrying the normal allelomorph for bobbed ( $+^{bb}$ ), a locus near the right end. More than 11,750 female offspring of females heterozygous for  $y$ -closed and for  $bb$ , mated to  $bb$  males, have been examined and none showed recombination of the extreme end characters. A stock, kept by mass cultures, of females heterozygous for  $y$ -closed and for bobbed mated to bobbed males was made from material from which the closed chromosome had always been selected by selection of females carrying the gene for yellow. For three years no recombination of the loci of  $y$  and  $+^{bb}$  occurred. Other stocks not marked for bobbed show linkage of the characteristics of the closed chromosome to yellow, for by selection of yellow, the closed chromosome has been kept since it was first found in 1922.

In order to test the closeness of the point of union to the gene for yellow, flies were made heterozygous for yellow carried by the closed chromosome and for broad (locus 0.6), or for prune (locus 1.0) or for allelomorphs of white (locus 1.5). No crossing over occurred between yellow and the locus for broad (among 6568 flies) or between yellow and the locus for prune (among 2138 flies) but 87 flies have been obtained that were crossovers between the loci of yellow and white. Of these, 25 were bred and all of them showed that the closed characteristics remained with yellow. Therefore, the factor or condition responsible for the chromosome being closed is so far to the left in the chromosome that 100 percent of 25 random samples of crossovers between 0 and 1.5 showed the locus of the factor to be to the left of the break.

Similarly the linkage of the point of union to the locus for bobbed was tested; 113 flies were obtained that were crossovers between carnation (4.5 units to the left of bobbed) and bobbed (near the end of the chromosome). Of many that were mated, 15 bred and showed that the characteristics of the closed chromosome accompanied the not-bobbed gene of the closed chromosome in each case, that is, the chromosome is closed by the attachment of the distal end to a point near the right end of the chromosome.

The genetic results showing the union of the ends of the chromosome to each other (locus of yellow to locus of bobbed) and the linkage of the point of union to the left end and to the right end of the chromosome are evidence for the hypothesis that the ends are physically attached and that the attachment is the condition whose effects are the characteristics peculiar to the closed chromosome.

It has been stated that a stock heterozygous for  $y$ -closed and for  $bb$  was maintained for three years by selecting females heterozygous for yellow, all of which were also not-bobbed. The chromosome carrying bobbed was also marked by scute and no scute not-bobbed female had ever been observed. At the end of that time there appeared scute not-bobbed females and also the percentage of yellow males increased, a class which had been extremely small up to that time, owing partly to inviability when the X chromosome is closed. The chromosome carrying yellow was tested and now gave among 1721 flies close to the standard percentages of both single and double crossovers that are regularly found between normal Xs, and did not produce the high percentage of patroclinous males which is found when a closed chromosome is involved. The chromosome was no longer distinguishable from a normal X.

Since the closed  $X^0$  was derived from attached XX, the reopening of the closed  $X^0$  suggests that it might have originated by the separation of a single X from attached XX, but this is not a satisfactory explanation, for,



if opening of the closed  $X^0$  could occur in that way, it would have taken place frequently instead of only once.

Flies carrying a closed  $X^0$  chromosome are poorly viable, and homozygous females and males show low fertility and sometimes abnormalities of the eyes or legs. In the reopened  $X^0$  these peculiarities have disappeared, so they may be due to irregularities caused by the closed condition. Jagged wings often found in the presence of duplication of sections of the X chromosome have not been observed.

It has been suggested that the closed  $X^0$  may have originated as a translocation of the yellow end of one attached X to a point near the spindle fibre attachment of the other X, or as a complete inversion in one attached X followed by single crossing over with the other. But both hypotheses imply the presence of a slight duplication at the spindle fibre end and leave open the possibility of release of the yellow end as when a detached X is derived from attached XX.

#### DOUBLE CROSSOVERS

The crossovers which are found among the offspring of a female carrying one or two closed chromosomes prove, when the chromosomes are completely marked, to be exclusively doubles or quadruples. It has been shown that among at least 11,750 flies from heterozygous mothers and in various heterozygous stocks those chromosomes that retained the left end of the closed chromosome carrying yellow also retained the right end carrying not-bobbed, and this linkage can be assumed to hold for flies that cannot be rated for bobbed. (In the first count of experiment A<sub>1</sub> two flies are recorded as recombinations of yellow and bobbed. Subsequently, whenever there was doubt as to the classification of bobbed, the fly was tested and among those that bred none showed recombination. The two exceptions had almost certainly been wrongly classified.) Using this assumption in classifying it was found that among 16,914 flies (table 10), all the crossovers, of which there were 2,211, were doubles or quadruples and none were singles or triples.

The genetic results show that if interchange takes place between a closed  $X^0$  chromatid and a normal X chromatid simultaneously at two points, viable zygotes of both complementary classes may reach the adult stage.

#### FREQUENCY OF DOUBLE CROSSOVERS

The attachment of the ends of an X chromosome affords a new situation for crossing over since single crossovers are not represented by the usual classes. It is of interest to know whether there is an effect on the frequency of double crossing over for the whole chromosome and for different regions of the chromosome. Frequencies of double recombinations have been determined by the method of alternated backcross devised by

BRIDGES and OLBRYCHT (1926) in mapping the X chromosome. Their results will be referred to as the alternated X-ple or a.X. values. The chromosomes used by them were so marked that crossing over could be followed in six regions, from scute at 0 to forked at 62 with little or no chance of double crossing over between the marked loci. The character bobbed near the extreme right end of the chromosome was not included but when the experiments with the closed chromosome were made, bobbed was introduced, thereby bringing under observation a seventh region of the chromosome to the right of forked.

Material descended from the a.X. stocks of BRIDGES and OLBRYCHT was used to make stocks heterozygous for  $X^0 y cv v f$  and for  $X^0 y ec ct^6 g^2$  and also to make the apposers and the testers that were used. The symbol  $X^0$  is used to designate the closed chromosome.

The combined experiments with marked flies heterozygous for closed  $X^0$  involved 16,914 flies not including those of non-disjunctional classes. As already stated, yellow in the closed chromosome is always linked to not-bobbed so that among males in which bobbed does not show, because of the normal allelomorph of bobbed carried in the Y chromosome, and among flies of experiment B<sub>2</sub>, where the tester was not bobbed, all yellow chromosomes were rated as not-bobbed, and not-yellow as bobbed. The apparent single crossovers were in this way found to be doubles that involved the seventh region.

It is apparent from the counts of observed offspring of heterozygotes (appendix, table 10) that factors for differential viability are present. Flies containing a closed  $X^0$  (yellow males and females heterozygous for yellow) occur less frequently than the complementary types, class for class, and furthermore the difference is proportionately greater among crossovers than among non-crossovers. The ratio of the frequency of non-crossovers of  $X^0$  classes to non-crossovers of X classes in the combined experiments is 73 to 100, and for crossovers the ratio is 29 to 100 in bottles containing a single female parent.

Viability probably varies with the conditions of the environment. When only the eggs laid by one heterozygous female in one day developed in a bottle, the ratio of  $X^0$  to X non-crossovers in one experiment was 89 to 100. In mass cultures of heterozygous females back-crossed to normal X males, there are few if any yellow males whereas with uniform viability they are expected to equal nearly 1/4 of the population. Homozygous cultures hardly survive even under favorable conditions.

The mortality occurs partly at least in the pupal stage. Of 62 dead pupae found in pair cultures of heterozygous females, 87 percent belonged to the classes carrying a closed  $X^0$ ; they were either  $X^0$  males or females heterozygous for  $X^0$ .

In the first two experiments ( $A_1$  and  $A_2$ )  $y\ cv\ v\ f$  females heterozygous for  $X^0y$  and  $sc$  were mated to  $ec\ ct^0\ g^2\ bb$  males, with or without scute, and  $F_1$  females were mated singly to  $X^0$  males which carried all of the recessive genes except scute. Cultures that gave yellow males in  $F_2$  are included in table 10 and those that gave not-yellow (or scute) sons were used as controls (appendix, table 11). The control involved a total of only 2,623 flies, but recombination percents (table 2) corresponded well enough with the a.X. values to show that the stock had not changed since it had been used by BRIDGES and OLBRYCHT, and more significant results of the larger a.X. experiment could be used. The map distances derived from the a.X. values are not the same as the present standard values for the X chromosome which have been more recently obtained from a wide range of material, but since the stocks used in the experiments were made from alternated X-ple material, the a.X. values are suitable for comparison.

TABLE 2

*Recombination values of the eight chief characters used in the experiments with the closed chromosome obtained from two control experiments and from "a.X." data of BRIDGES and OLBRYCHT.*

	REGION							TOTAL NUMBER OF FLIES
	$ec-ec$ 1	$ec-cv$ 2	$cv-ct^0$ 3	$ct^0-y$ 4	$y-g$ 5	$g-f$ 6	$f-bb$ 7	
From a.X.	6.8	9.6	8.2	14.8	11	11.4		20,786
From two		10.6	8.3	16.2	11.1	12.7	10.5	2,623
controls	7.2							585

In order to compare the rates of crossing over in different regions of the chromosome the total percent of recombination observed in each region (appendix, tables 10 and 12) has been divided by the number of the a.X. map units that represent the length of the region. The horizontal axis of diagram 1 represents the chromosome map, the vertical axis the frequency of crossing over expressed as percent per map unit of the total number of observed recombinations for each region. In order to eliminate the factors for differential viability among offspring of heterozygotes, the percents of recombination have been calculated separately for offspring of the two classes, with and without a closed  $X^0$ .

The frequency of crossing over in the first and seventh regions of flies homozygous for closed  $X^0$  cannot be observed directly because no new characters can be introduced at the ends of the chromosome; double cross-overs involving either of the two end regions therefore appeared to be singles and the doubles involving both end regions appeared to be non-crossovers. The percent of apparent singles from females homozygous for closed  $X^0$  is 3.42. The percent of crossovers involving either the first or the seventh region among flies that received the closed  $X^0$  from hetero-

*zygous* mothers is 3.44, a close agreement for both regions together. In default of direct values for doubles involving these regions, the singles have been allocated as doubles in the same proportions as were found in experiments A<sub>1</sub> and A<sub>2</sub>, in which the closed X<sup>0</sup> came from heterozygotes. Similarly a certain percentage of phenotypical non-crossovers from homozygotes, corresponding to the percentage of crossovers from heterozygotes involving both the first and seventh regions, have been rated as crossovers of the 1, 7 class. The percentages obtained are nearly equal to the percentages that closed X<sup>0</sup> crossovers are of the closed X<sup>0</sup> class from heterozygotes.

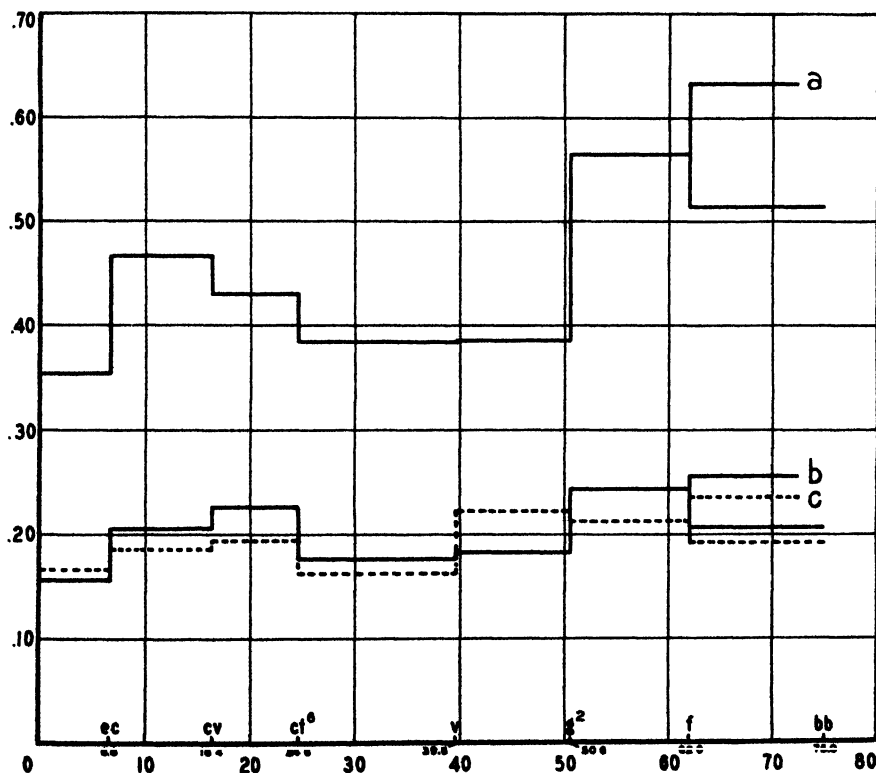


DIAGRAM 1.—Frequency of double crossing over found in flies marked for seven regions of the X chromosome. (a) Percents per map unit of double crossovers from females heterozygous for closed X<sup>0</sup> as shown by offspring not carrying a closed X<sup>0</sup>. (b) Same from offspring carrying a closed X<sup>0</sup>. (c) Percent per map unit among offspring of females homozygous for closed X<sup>0</sup>.

The ratio of the percent of crossing over among flies of the closed X<sup>0</sup> class from heterozygotes to the percent of crossing over among the normal X class is a little less than 1:2 for every region of the chromosome. It will be shown later that this ratio is due in all probability to a lethal class among closed X<sup>0</sup> crossovers, and in the comparisons with normal crossing over that will follow, values for the normal X class will be used.

Crossing over in heterozygous and in homozygous  $X^0$  females (diagram 1) is lowest in the fourth region and increases on both sides; after a maximum in region 3 or 2, there is a decrease to the left. A low rate of crossing over at the left end of the  $X^0$  chromosome has been found, as already stated, in experiments with chromosomes marked close to yellow, for no recombination occurred of yellow and broad or yellow and prune.

It is difficult to determine the percent of crossing over per unit of map distance at the right end because the distance from forked to the end of the chromosome was not known for the a.X. chromosome, and that distance has been found from unrelated experiments to be very variable. If the value of about 13 units found by STERN (1926) is used, the percent per unit drops for the seventh region, but, if 10.55 units found in the small control experiment is used, the curves rise. In experiment  $B_1$  and  $B_2$  when a new dominant character Beadex-2, 3.3 units to the right of forked, was introduced into the apposing chromosome, the drop found for 13 units from forked to bobbed was progressive from forked to beadex to bobbed. The most recent values for forked to beadex and beadex to bobbed obtained from many different stocks are 2.6 and 6.6 respectively. Using these values the percent of crossing over per unit increases progressively from the fourth region to the right end of the chromosome.

#### COMPARISON WITH A NORMAL X CHROMOSOME

In order to make percentages derived from alternated X-ple values comparable with those from experiments with closed  $X^0$ , some corrections are necessary. Assuming that single crossovers with the closed chromosome are inviable, only double crossover frequencies for two normal Xs can be compared with the crossover frequencies in females heterozygous for normal X and closed  $X^0$ ; accordingly, percentages of doubles from a.X. have been computed as of the total number of flies minus the singles and triples, except that the undetectable singles of the seventh region are not subtracted, which makes the percentages for a.X. too low. Furthermore, since in the a.X. experiments the seventh region was not marked, crossovers that were in fact doubles involving the seventh region appeared to be singles and have been subtracted as such from the grand total when singles have been subtracted. It is therefore necessary for comparison to subtract from the closed  $X^0$  totals all the doubles involving the seventh region. Exceptional classes have also been subtracted.

The results obtained from the corrected data are given in table 3. They show that the frequency of total double crossing over in heterozygotes, as indicated by the normal chromosomes that are recovered, is less than the frequency of double crossing over between normal Xs.

A more detailed comparison has been made for different regions of the chromosome. The curves of diagram 2 have been obtained from the corrected data showing the percents of total crossing over per map unit for six regions based on doubles and quadruples when the seventh region of the chromosome is not under observation; the percents for a.X. being as explained a little too low. Disregarding for the present the left end of

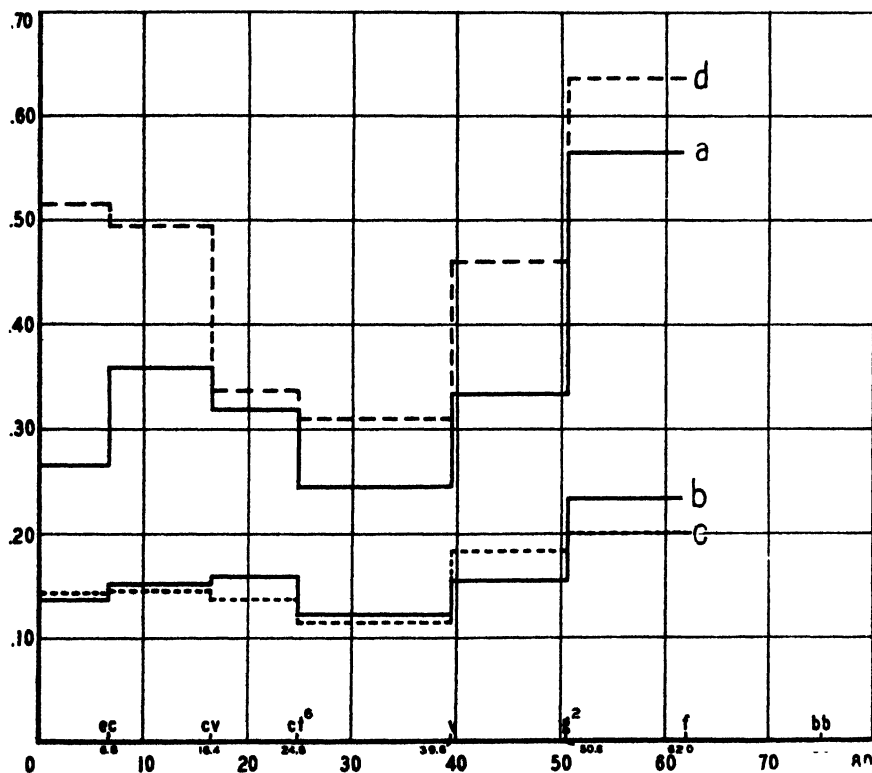


DIAGRAM 2.—Frequency of double crossing over with closed  $X^0$  from data corrected for comparison with alternated X-ple (a.X., BRIDGES and OLBRYCHT) in six regions of the X chromosome (a, b, c as in diagram 2, d percents per map unit of double crossovers from corrected data of a.X.)

the chromosome it is apparent that the percent of crossing over in the normal class from heterozygotes is for every region to the right of *cv* somewhat, though not constantly, lower than the percent of crossing over between normal Xs. From *cv* to *cl* the percents are most nearly equal, and the relative percents for heterozygotes fall progressively from *cl* to *g*. The percents for homozygous closed  $X^0$  from *cv* to *g* follow more closely the changes in percents for normal Xs, maintaining a nearly 1:3 ratio; this continues in the *g* to *f* region, in which the percent from heterozygotes again approaches more nearly the normal X value. The result may be stated in another way; the ratios of the frequencies of double crossovers

from two normal chromosomes and from a closed and a normal chromosome vary in different parts of the chromosome from *cv* to *f*, while the relative frequencies of double crossovers from chromosomes that are alike, either both normal or both closed, are more constant from *cv* to *f*.

TABLE 3

*Relative percents of crossovers from females heterozygous for closed  $X^0$  and from females homozygous for normal  $X$  or for closed  $X^0$ .*

	CORRECTED TOTAL ADULT FLIES		PERCENT OF DOUBLE CROSS- OVERS OF SIX REGIONS AS OF CORRECTED TOTAL	RATIO OF PERCENT OF DOUBLE CROSSES FROM $X^0/X$ TO PERCENT FROM $X/X$	TOTAL OBSERVED REGULAR ADULT CLASSES	PERCENT OF DOUBLE CROSS- OVERS OF SEVEN REGIONS AS OF TOTAL OBSERVED REGULAR ADULT CLASSES	TOTAL POPULATION	PERCENT OF SINGLE CROSS- OVERS AS OF TOTAL POPULA- TION	RATIO OF PERCENT OF SINGLE CROSSOVER CHROMATIDS FROM $X^0/X$ TO PERCENT FROM $X/X$
	SEVENTH	FIRST							
a.X. $\frac{X}{X}$	11111*		14.0*				20786	46.7	
$X^0/X$ $\frac{X}{X^0}$	9488		10.8	0.77					
	6564		4.95	0.35					
Homozygous $X^0$	2580		4.76						
Control $\frac{X}{X}$	1420*		20.7*						
$X^0/X$ $\frac{X}{X^0}$	9924		14.72	0.71	10168	16.7			
	6674		6.54	0.32	6746	7.54			
Homozygous $X^0$	2616		6.46		2646	7.1			
Exp. $J_2$ $X^0/X$ $\frac{X}{X^0}$					1928	20.4	3135+	30.8+	0.66
					1438	9.4			

\* Corrected totals for a.X. and control are too high because they include undetectable singles of the seventh and first regions respectively; the percents are correspondingly too low (see text).

+ Computed for first 6 days of laying (see table 7).

For the seventh region the percents of crossing over for the closed chromosome were directly compared with those from the small control experiment, which had been made to check the material for a.X. values (see table 3). In the control, the first region was not marked and corrections were made similar to those made for comparison with the a.X chromosome, unmarked in the seventh region. Again the percents from the control are a little too low owing to undetectable singles of the first region being included in the total. This does not affect the conclusion that there is no relative decrease in crossing over at the right end of the closed  $X^0$  (table 4). Ratios for the normal  $X$  class from heterozygotes show a decrease for the *v-g* region consistent with that found in the comparison with a.X. (diagram 2) and an increase toward the right end. The results for closed  $X^0$  classes are not entirely consistent.

TABLE 4

*Ratios of the percents of crossing over in regions 2 to 7 in flies heterozygous and homozygous for closed X<sup>0</sup> to the percents of crossing over obtained from a small control experiment. The percents are obtained from corrected data and are based on the frequency of observed double and quadruple cross-*

		y-ec	ec-cv	cv-cl	cl-s	s-g	g-f	f-bb
X/X <sup>0</sup>	X		0.60	0.72	0.72	0.66	0.71	0.80
	X <sup>0</sup>		0.27	0.38	0.31	0.38	0.29	0.34
Homozygous X <sup>0</sup>			0.23	0.32	0.28	0.32	0.26	0.21

A reduction in crossing over at the left end of the chromosome beginning in the second or first region was found to occur with a closed X<sup>0</sup> when all regions were included (diagram 1), and appears also when the cross-overs involving the seventh region are not included (diagram 2). Crossing over between two normal Xs on the other hand increases progressively from the region that is the minimum for all the chromosomes, continuously, to the extreme left end of the chromosome.

The attachment of the left end of the closed chromosome to the right end is in a sense a translocation, and, as in translocations generally, there is a decrease in crossing over near the region of attachment; but this is not a reduction due to the conflict of attractions of genes of the sort described by DOBZHANSKY (1931) in the true translocations. The X chromosomes are intact and neither chromosome is attracted to parts of another that are controlled by two different spindle fibres as in cases cited by him. The results are in accord with the view of BEADLE (1932) and of OFFERMAN and MULLER (1932) (see also GRAUBARD 1931) that proximity to the spindle fibre attachment is a factor that reduces crossing over, for in the closed chromosome the attachment of the yellow to the bobbed locus has brought the left end of the chromosome near to the spindle fibre attachment.

#### INVIALE CROSSOVERS

Crossing over, or exchange of segments between chromatids, takes place in *Drosophila* in the four-strand stage (BRIDGES 1916, ANDERSON 1925, L. V. MORGAN 1925, STURTEVANT 1931). In females heterozygous for closed X<sup>0</sup> and normal X two chromatids have no free end which imposes a new condition on the recovery of crossovers. For, if a closed and a normal chromatid exchanged segments at one level only (diagram 3, S), the result would be two tandem chromatids, attached together, because the "yellow" or distal end of the original closed chromatid is inseparable from its spindle fibre end; the chromatid complex would have a spindle fibre attachment at one end (derived from the normal X) and a second attachment (derived from the originally closed X<sup>0</sup>) in the middle of the new double chromatid.



If the double complex passes entirely into one nucleus, non-disjunctional females with united Xs would be expected. Non-disjunctional females are not unusually frequent and a few that were tested contained separate X chromosomes, not a double complex. It is therefore assumed that a complex carrying two spindle fibre attachments may be lethal and some evidence for the assumption will be given later. DOBZHANSKY (1931) has pointed out that among translocations some would be expected leading to the formation of chromosomes having more than one spindle fibre attachment and there are no descriptions of such chromosomes in the literature.

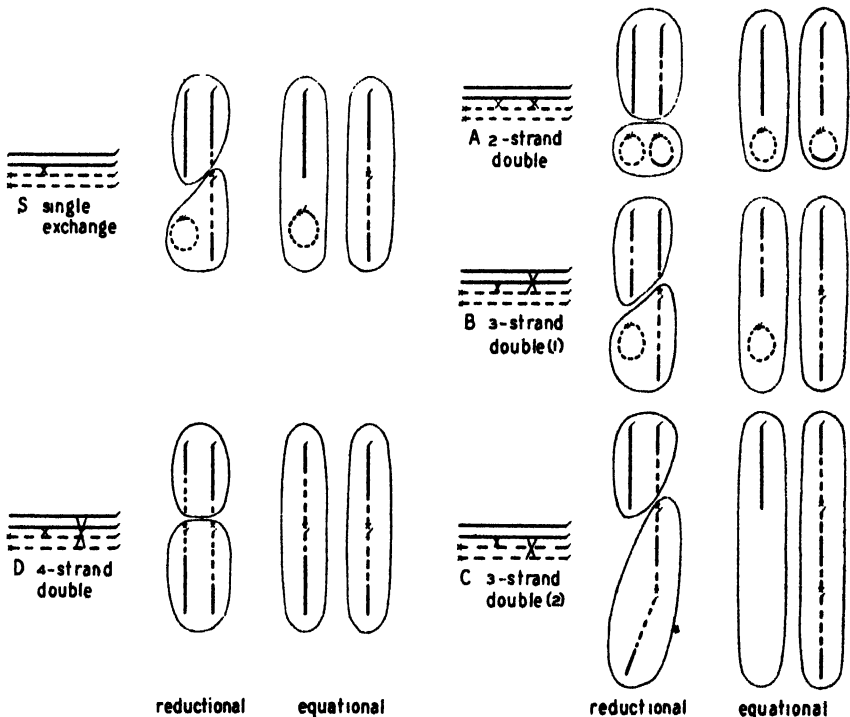


DIAGRAM 3.—Analysis of chromatid formation and distribution at the first meiotic division, when exchanges between homologues have taken place.

Double exchange (that is, two exchanges at the same time, not at the same level [ANDERSON, 1929]) may involve two or three or all four of the chromatids at once. Diagram 3 represents the four possible second exchanges between homologous strands that may occur simultaneously with a first exchange between homologues. A 2-strand double exchange leaves all the chromatids separate from one another and may result in a non-crossover and a double crossover of each kind, closed and normal (diagram 3A). The term normal designates a chromatid which contains the spindle fibre end derived from a normal chromosome.

A 4-strand double exchange is equivalent to two simultaneous single exchanges (diagram 3D) and is by hypothesis therefore lethal for all four chromatids.

A 3-strand double exchange may be one of two kinds. If two of the three strands are normal chromatids (each with a free end, diagram 3B) there may be recovered a closed  $X^0$  non-crossover and a normal X double crossover since the exchanges lead from one normal spindle fibre end to the free end of the other normal chromatid. From the other normal spindle fibre end, however, exchange leads to the end of the closed chromatid attached to its own spindle fibre end, and the result is a lethal double complex.

If two of the three exchanging strands are closed chromatids one non-crossover normal chromatid may be recovered, but the other three chromatids will all be united (diagram 3C) and will *a fortiori* be lethal.

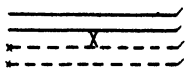
Closed  $X^0$  double crossovers can therefore be derived from 2-strand double exchange only, but normal double crossovers are derived from both 2-strand and 3-strand exchange. It has been noted that the ratio of the percent of double crossovers among closed  $X^0$   $F_1$ s to the percent of double crossovers among normal X  $F_1$ s is very nearly the 1:2 ratio to be expected if exchange between homologous strands is at random.

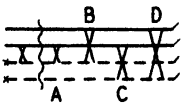
The 1:2 ratio for closed  $X^0$  to normal X double crossovers shows that homologous strands cross at random when two or three strands are involved in double exchange. The closed  $X^0$  tells nothing about the frequency of 4-strand double exchange but EMERSON and BEADLE (in press) have found from data for attached X that 2-strand and 4-strand double exchanges are equally frequent. This result and the equality between 2-strand and 3-strand exchanges shown by closed  $X^0$  are to be expected if when there is crossing over at one level a second crossing over at another level takes place by random exchange of homologous chromatids. The expectation is the same if triple exchange is random (see diagram 4 and table 5). It may be concluded therefore that exchange between homologous chromatids is at random.

#### PATROCLINOUS MALES

Patroclinous males occur in the proportion of 1 to 3 normal X double crossovers. This is chosen as a critical ratio because of the good viability of the normal class and because almost all double crossovers are derived from double exchange which perhaps is the source also of almost all patroclinous males. It has been found that closed  $X^0$  double crossovers are half as frequent as normal X doubles and it has been shown that half of the closed  $X^0$  doubles expected by random exchange between homologous chromatids are probably inviable. An analysis of the distribution of chromatids shows that patroclinous males may arise at the expense of

closed  $X^0$  doubles. A closed  $X^0$  double crossover from 3-strand double exchange involving two closed  $X^0$  chromatids (diagram 3C) would be inviable because it would be united with two other chromatids, in a lethal triple complex. If at the first meiotic division two of the chromatids that are going together to one pole (either equationally or reductionally) drag the third chromatid away from the other pole, one daughter nucleus might

	non - c o		c. o.		no - X	invi- able
	X <sup>0</sup>	X	X <sup>0</sup>	X	$\frac{1}{2}$	
single exchange						2
						
ratio	1:1					

double exchanges						2 2 $\frac{1}{2}$
						
ratios	2:2		1:2		4:1	8 $\frac{1}{2}$

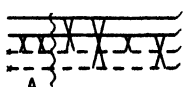
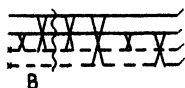
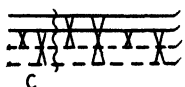
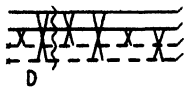

triple exchanges						8
						
						8 $\frac{1}{2}$
						
						10 $\frac{1}{2}$
						
ratios	4:4		6:12		2	36

DIAGRAM 4.—Chromatids and ratios to be expected from random exchanges between homologous chromatids in heterozygotes for closed  $X^0$  and normal X, on the assumptions that double and triple complexes are lethal and that patroclinous males are mainly derived from double exchange C. A closed chromatid is represented by a broken line and the end marked "x" is attached near the spindle fibre to the same chromatid. On the right of the wavy lines are represented the different exchanges that might occur simultaneously with the exchange or exchanges on the left of the line, by double and triple exchange.

contain only one X chromatid and at the second division one no X nucleus might be formed. If such a nucleus remained in the egg, it would, if fertilized by Y sperm, be lethal in the egg stage according to LI (1927), and if fertilized by X sperm it would contain the chromosome complement

of a patroclinous male. If patroclinous males have this origin the expected frequency from random double exchange is 1 to 4 normal X doubles, which is lower than the observed frequency of 1 to 3. (From infrequent triple exchange the expectation, if patroclinous males are due to formation of triple complexes, is still lower; see diagram 4 and table 5).

TABLE 5

*Classes and Ratios; (1) To be expected (a) If homologous strands of closed X<sup>0</sup> and normal X cross at random and if patroclinous males are derived from one kind of 3-strand double exchange, and united chromatids produce inviable eggs.*

TYPE OF EXCHANGE		NON-CROSSOVERS X <sup>o</sup> : X		DOUBLE CROSSOVERS X <sup>o</sup> : X		NO X EGGS BY X SPERM (PATROCLINOUS MALES)	INVARIABLE EGGS	RATIO OF X NON-CROSSOVERS TO INVARIABLE EGGS
none		1	1	0	0		0	
	ratio	1:1						1:0
single		1	1	0	0		2	
	ratio	1:1						1:2
double		2	2	1	2	0.5	8.5	
	ratio	1:1		1:2		4:1		1:4
triple		4	4	6	12	2	36	
	ratio	1:1		1:2		6:1		1:9
(b) If all strands cross at random								
none		1	1				0	
	ratio	1:1						1:0
single		6	8	0	0		10	
	ratio	75:100						0.8:1
double		10	16	1	2	2.5	28.5	
	ratio	62:100		1:2		4:5		1:2
triple		19	39	19	29	11	139	
	ratio	50:100		2:3		3:1		1:3
(2) Observed								
	ratio	1:1		1:2		3:1		1:1.2

The source of some of the patroclinous males may therefore be looked for in other kinds of exchanges and although little is known about the separation of chromatids at meiosis in *Drosophila* a formal analysis can be made, presenting at least some of the problems involved.

Two-strand double exchange (diagram 3A) results in four single chromatids which may be distributed regularly among the four maturation nuclei. Four-strand double exchange (diagram 3D) results in two double



If the reductional division takes place first, each daughter nucleus will contain one of the single chromatids resulting from exchanges S or B, and if these single chromatids are to be recovered the nuclei must be viable. The first division can be truly reductional (that is, the sister chromatids can remain together at the fibre end) only in case the normal component of the double complex is torn apart, and the complex is not lethal in the sense that has been assumed (see diagram 3 and 5). If chromatids are severed in this way, one of the daughter nuclei at the second (equational) division might receive the normal X chromatid and the other might receive a fragment or fragments of a chromatid; a nucleus with a fragment and its spindle fibre attachment might produce an inviable zygote or, if fertilized by X sperm, might produce a female with a deficiency in one of her X chromosomes. No such flies have been observed. If a fragment with a spindle fibre attachment were small, and sometimes went with the whole chromatid at the second division, duplications would be expected among the offspring, but these were not found. If all fragments were lost a nucleus containing no X chromatid might contribute to the class of patroclinous males.

The other daughter nucleus of the first reductional division would contain the spindle fibre ends of the closed chromatids (diagram 3S); one chromatid would be a closed non-crossover and the other chromatid might carry an extra piece torn from the normal chromatid that had become united to it by crossing over. The second division would give a nucleus with a closed chromatid, and one of a kind that would produce inviable zygotes or females with duplications, which are not found. If the united chromatids separated at the first division at their point of attachment single crossovers with the fibre attachment of the original closed  $X^0$  would be expected but are not found (diagram 5, scheme 1). Similarly duplications and single crossovers might sometimes be expected from 4-strand double exchange (diagram 3D).

Three strand exchange that involves two closed  $X^0$  chromatids (diagram 3C) and that results in a normal X chromatid and three chromatids united together might be expected to produce similar exceptional classes by rupture of chromatids if the first division is reductional. Or the three united chromatids might all go into one nucleus, giving the results already formulated.

If however the first meiotic division were equational, the recovery of single chromatids after exchanges S and B would involve no united chromatids whenever the double complex segregated from the two single chromatids (diagram 5, scheme 4). Assuming that double complexes are lethal no viable chromatids would be expected after 4-strand double exchange with this kind of equational division.

But on chance association of spindle fibre ends, the ends of a free and of an attached chromatid would go together at a first equational division as frequently as the ends of the free chromatids would separate from the ends of the attached chromatids. The resulting nuclei after the second division would be of the same kinds as after a first reductional division (see diagram 5, scheme 1) and classes would be expected that have not been observed.

If all four X chromatids went into one first daughter nucleus and no X into the other, the first division would be neither equational nor reductional. Following out such a possibility to the second division the no X nucleus might divide into two no X nuclei and the other nucleus divide also. But whether such a nucleus underwent the second division reductionally (diagram 5, scheme 5) or in either of two ways equationally (diagram 5, scheme 2 or 3) non-disjunctional and other classes that have not been observed should result.

It appears that the kind of assortment of chromatids that best fits the observed classes, except for the excess of patroclinous males, is one which segregates the free chromatids from a double complex at the first division (diagram 5, scheme 4).

If exceptionally, at the first division, two united chromatids and only one of the single chromatids went together into one nucleus and the single chromatid separated from the double complex at the second division (diagram 5, scheme 6), the 1:1 ratio between non-crossovers and the 1:2 ratio between double crossovers would be maintained and there would be an increase in the frequency of no X eggs, and therefore of patroclinous males, derived from one half of the nuclei that after the second division contained no X.

The observed percent of double crossovers from homozygous closed  $X^0$  females is in agreement with expectation on the assumptions that have been made from a study of heterozygotes. Two-strand exchange would give viable double crossovers as in heterozygotes and 3-strand exchanges would produce patroclinous males. The double crossovers recovered should therefore be the same percent of the total regular classes as are closed  $X^0$  doubles of the total closed  $X^0$  class from heterozygotes which was realized (table 3 and diagrams 1 and 2). The percent of patroclinous males from homozygotes, if they are derived mostly from 3-strand exchange, would be expected to equal the percent of patroclinous males as of a total consisting of the closed  $X^0$  class plus the patroclinous males from heterozygotes. Homozygotes gave 12 percent among a total of 2121 flies (table 12). Heterozygotes of experiment Aa gave 10 percent among 2087 flies of the corresponding classes and 9 percent occurred among another total of 2588. This is a close agreement but viability of the closed

$X^0$  class as compared with that of the normal X class was exceptionally low in both the experiments with heterozygotes. In another experiment ( $B_1$ , table 10) in which viability of closed  $X^0$  flies as compared with viability of normal X flies was almost twice as good, patroclinous males were only 5.6 percent of a corresponding total of 1534. There was no measured control of the viability of  $F_1$ s from homozygotes but among 3 cultures there was an average of only 62 flies of regular classes from a culture; larvae were numerous, but not pupae and many of the pupae were dead; also many flies that hatched did not survive. It may be concluded that, although differential viability obscures the result, the unexpectedly high percentage (12 percent) of patroclinous males from homozygotes may not be at variance with the assumption that they originate mainly from eggs in which triple chromatid complexes are formed by crossing over.

#### INVIABLE EGGS

In *Drosophila*, the first meiotic division is not completed until after the sperm has entered the egg (HUETTNER 1924, GUYENOT and NAVILLE 1929) and since crossing over takes place at the four strand stage, which occurs at meiosis, irregularities due to crossing over should affect the egg stage (see PLOUGH 1921, GOWEN 1929a and b). It follows that a certain proportion of the eggs laid by flies heterozygous for closed  $X^0$  and normal X would be expected to be inviable if double or triple crossover complexes are lethal as has been assumed, but that if crossing over were reduced, or prevented, a corresponding reduction in inviable eggs would be expected.

Dr. JACK SCHULTZ suggested a comparison of the viability of eggs from females heterozygous for closed  $X^0$  and normal X with viability of eggs from females in which the normal X has been replaced by an inverted X called  $\Delta$ -49 (MULLER and STONE 1930, OFFERMAN and MULLER 1932) preventing nearly all crossing over.

The flies used were  $X^0 y/g^2$  females mated to  $y g^2 bb$  males,  $X^0 y/\Delta$ -49  $cm \pm bb$  females mated to  $y \Delta$ -49  $cm bb$  males, and for control  $\Delta$ -49/ $g^2$  females by  $g^2$  males. A single female was put into a vial with many males, and the next day transferred to a bottle in which was placed food darkened by lampblack and contained in the depressions of handles of two paper spoons. On the first day following and on five successive days the spoons were removed and new spoons put into the bottle with the flies; the eggs on the spoons that had remained a day with the flies were counted and the spoons put into new bottles supplied in the regular way with fly food. On the fourth day eggs still unhatched were counted. Eight females of each kind were mated and the eggs that were laid on the first six days were followed in this way.

The result is consistent with the hypothesis for the percent of inviable eggs was reduced from 31.3 percent to 6.3 percent when crossing over with



a closed  $X^0$  was reduced by the inversion in the  $\Delta$ -49 chromosome (see table 6).

TABLE 6

*Percent of classes representing crossovers from females carrying inverted and closed  $X^0$  chromosomes (deduced values in italics).*

EXPERIMENT	HETEROZYGOTE	PATROCLINOUS $\sigma^2$	SINGLE CROSSOVER	DOUBLE CROSSOVER	INVIABLE EGGS	TOTAL
A.H.S. (unp.)	CIB/X	0.5				1743
	CIB/X		0.5			
C	CIB/ $X^0$	0.52		0.1		6122
L <sub>1</sub>	$\Delta$ -49/X	0.27				1798
L <sub>2</sub>	$\Delta$ -49/ $X^0$	0.33				4150
K <sub>1</sub>	$\Delta$ -49/ $X^0$	0.5				2099
M	$\Delta$ -49/X	0.3				1329
J.S. (unp.)	$\Delta$ -49/X		13.0	0.4		1309
J <sub>2</sub>	$\Delta$ -49/X				3.2	3498
J <sub>1</sub>	$\Delta$ -49/ $X^0$			0.0	6.3	3383
J <sub>2</sub>	$X^0$ /X		0.0		31.3	3326
		<i>2.36</i>		<i>14.3</i>	<i>33.2</i>	<i>3135</i>

Furthermore the presence of some inviable eggs from heterozygotes for closed  $X^0$  and  $\Delta$ -49 is consistent with the conclusion reached from analysis of maturation divisions that usually two single chromatids segregate from a double lethal complex. Dr. SCHULTZ found that heterozygotes for  $\Delta$ -49 and normal X gave 13 percent of single crossovers due to single exchange in the region not included in the inversion of the  $\Delta$ -49 chromosome, and only 0.4 percent of doubles (which involved the left end of the chromosome). The kind of segregation cited has been shown to be the only scheme on which inviable eggs would regularly be produced by single exchanges (see diagram 5). Since  $\Delta$ -49 gave 3.2 percent of inviable eggs (due to unknown causes) in the control with normal X (experiment J<sub>2</sub>, table 6), it may seem that 6.3 percent of inviable eggs from heterozygotes for  $\Delta$ -49 and closed  $X^0$  is not high enough to correspond with 13 percent of single crossovers from heterozygotes for  $\Delta$ -49 and normal X; that they do correspond is however the most consistent conclusion especially since single crossover chromatids from closed  $X^0$  and normal X seem to be only 0.66 times as frequent as single crossovers from normal Xs.

A second conclusion from analysis of meiotic divisions, namely, that patriclinous males mostly result from double exchange, is further shown by the percents of patriclinous males found from flies heterozygous for closed  $X^0$  and for  $\Delta$ -49 or CIB, another longer inversion.

The frequency of non-disjunctional classes, especially of males is known to be high among offspring of flies heterozygous for inversion and normal X (see table 6). For example in experiment L,  $\Delta$ -49/X females gave 0.27

percent of patroclinous males, and CIB/X females gave 0.5 percent as computed from unpublished data of Dr. STURTEVANT. Flies heterozygous for closed  $X^0$  and the inversion  $\Delta-49$  gave 0.33 percent in the controlled experiment L, and flies heterozygous for  $X^0$  and CIB gave 0.52 percent; that is when inversions are present closed  $X^0$  gives the percents of patroclinous males that are normal for the inversions and not the high percents that are characteristic for heterozygotes of closed  $X^0$  and normal X. The percent of all crossovers from CIB/X females is said to be not more than 0.5 percent and CIB/ $X^0$  females gave only 0.09 percent of double crossovers. The reduction of double crossovers by the inversion of a chromosome is accompanied by decrease of patroclinous males among offspring of flies carrying closed  $X^0$ . That patroclinous males are seldom derived from single exchange is indicated because there is no increase in their percentage from closed  $X^0/\Delta-49$  females as compared with that from normal X/ $\Delta-49$  females although some single exchange occurs with the inversion  $\Delta-49$ .

The results just cited in regard to patroclinous males and inviable eggs obtained from flies heterozygous for closed  $X^0$  and inversions, and the results from flies heterozygous for closed  $X^0$  and normal X, namely the absence of increase in the frequency of non-disjunctional females, the absence of a class with united Xs, and of duplications and deficiencies, the near agreement between the frequency of patroclinous males and  $1/4$  of the frequency of normal X double crossovers, suggest that in eggs containing closed  $X^0$  there is usually segregation of single chromatids from united chromatids at the first maturation division when two chromatids become united by single exchange. Such a segregation is equivalent to one kind of equational division.

#### FREQUENCIES OF ALL CLASSES

Whatever the mechanism by which exchange between chromatids produces lethal effects in flies heterozygous for closed  $X^0$  the inviable eggs and patroclinous males together should, on the hypothesis that inviability of eggs is due to lethal effects of crossing over, correspond to single crossovers, triple crossovers, and about  $1/4$  of the double crossovers from normal Xs. Patroclinous males were 2.36 percent of the deduced total population in the egg count experiment, and inviable eggs were 33.2 percent of that total (see table 7); the classes enumerated are therefore 35.56 percent of the total, a value which is low compared with normal X; but it is of the order to be expected, and is in agreement with other results with closed  $X^0$  that show a lower rate of crossing over than that which occurs between normal Xs. From corrected data for alternated X-ple, the percent of doubles among the normal X offspring of closed  $X^0$  heterozygotes is

0.77 times the percent of doubles from the a.X. control, and the percent of computed singles as of the whole population in the egg count experiment is 0.66 times the percent of singles in a.X. (see table 3).

Values from preliminary unpublished data recently obtained by Dr. BRIDGES in an experiment (425 C) with completely marked Xs are compared in table 8 with deduced values from the egg count experiment J<sub>2</sub>.

TABLE 7

*Observed and deduced frequencies of classes of experiment J<sub>2</sub>. Offspring of X<sup>0</sup>y/g<sup>8</sup> females by yg<sup>8</sup>bb males. Deduced values are in italics.*

Total no. of eggs 3326	adult flies	X	non-crossovers	849	27.1 percent
			doubles	224	7.15 percent
		X <sup>0</sup>	non-crossovers	754 = 89 percent of 849	24.0 percent
			doubles	73 (89 percent of 112 = 99)	2.33 percent
		patro- clinous males	XO assumed to be mostly from 3-strand double exchange (2)	74 (74:224 = 33:100) (56:224 = 25:100) 56 may be expected from 3-strand double exchange (2)	2.36 percent
	in viable in late stages	X <sup>0</sup>	X <sup>0</sup> non-crossovers	95 (849-754)	3.03 percent
			X <sup>0</sup> 2-strand crossovers	26 (on basis of viability of non-crossovers, 99-73)	0.83 percent
	in viable eggs	1040	in viable complexes from exchanges (single crossovers)	966	30.81 percent
			YO assumed to be of same origin and fre- quency as observed XO	74	2.36 percent
					3135
not accounted for from all classes				191	
				3326	

Non-crossovers from closed X<sup>0</sup> heterozygotes are 1.38 times as frequent as from normal flies. The deduced value for single crossovers in J<sub>2</sub> is 0.65 times the percent of singles from normal Xs. The percent of double crossovers in experiment J<sub>2</sub> is based on observed crossovers among flies carrying normal X; crossovers in experiment J<sub>2</sub> occurred with the maximum frequency that was observed among various experiments (see table 10,

appendix, and table 3); corrected for average frequency the value is 11.2. The consistently lower percentage of normal X doubles in every region of the chromosome in the more extensive experiments with heterozygotes as compared with alternated X-ple (diag. 3) seems to show that the true value for frequency of double crossovers from heterozygous  $X^0$  is lower than for flies carrying only normal Xs.

TABLE 8

*Percents of classes derived from females heterozygous for closed  $X^0$  and from normal females in two 6-day experiments. Percents of experiment 425 C are computed from preliminary unpublished data of C. B. Bridges on crossing over of completely marked X chromosomes. Deduced percents from experiment  $J_2$  are from table 7.*

EXPERIMENT	$P_1$	NON-CROSSOVER	SINGLE AND TRIPLE CROSSEVER S	DOUBLE AND QUADRUPLE CROSSEVER S	TOTAL
$J_2$	$X^0/X$	54.2	30.8	14.3 (11.2)*	3135
425 C	$X/X$	39.3	47.6	12.2	1243

\* Percent deduced from other experiments.

#### FREQUENCY OF MEIOSIS WITHOUT EXCHANGE

If the conclusion that homologous strands cross at random, reached by analysis of crossing over in attached XX and in closed  $X^0$  is applied to BRIDGES'S data for the normal X chromosome (table 9), it can be shown that some crossing over between normal chromatids takes place at meiosis in all eggs in *Drosophila*.

TABLE 9

*Percent of eggs of *Drosophila melanogaster* in which no exchange between chromatids of the X chromosome occurs at maturation, computed from observed crossovers from completely marked X chromosomes. (Observed values are from preliminary unpublished data of C. B. Bridges's experiment 425 C).*

	NON-CROSSOVER	SINGLE	DOUBLE	TRIPLE	QUADRUPLE	TOTAL
Number	1050	1332	330	17	2	2731
Percent	38.3	48.7	12.1	0.6		
	0.6	1.8	1.8			
	37.7	46.9	10.3			
	10.3	20.6				
	27.4	26.3				
	26.3					

1.1 percent of chromatids resulting from no exchange

0.25 percent of eggs in which no exchange takes place

If exchange takes place at random among normal homologous chromatids, 8 non-crossover to 24 single crossover to 24 double crossover to 8 triple crossover chromatids is the expectation from triple exchange. The observed percent of triple crossovers from completely marked chromosomes was 0.6 percent; if three times 0.6 is subtracted from 12.1 percent of observed double crossovers there remain 10.3 percent of doubles from double exchange. The expectation from double exchange is 4 non-crossover to 8 single crossover to 4 double crossover chromatids; if twice 10.3 (which is the expected percent of single crossover chromatids due to double exchange) and three times 0.6 (the percent of single crossover chromatids expected from triple exchange) are subtracted from the observed 48.7 percent of single crossovers, there remain 26.3 percent due to single exchange. An equal percent of non-crossover chromatids is to be expected due to single exchange. When all percents of non-crossovers due to triple, double, and single exchange have been subtracted from the observed percent of non-crossovers there remain only 1.1 percent of chromatids to be expected from 0.25 percent of eggs or no eggs in which maturation has taken place without any exchange between chromatids.

Since crossing over is less frequent in heterozygotes for closed  $X^0$  there may be some eggs in which no crossing over takes place.

The ratio of normal X non-crossovers to inviable eggs is expected by random exchange between homologous chromatids to be 1:2 as far as it is affected by single exchange, and 1:4 as far as affected by double exchange. The ratio will be increased in the proportion of 1:0 as far as no exchange occurs (tables 5 and 7). The observed ratio is 27:33, which is not inconsistent with expectation.

#### SISTER STRAND CROSSING OVER

Up to this point, ratios that have been examined have been based on exchange (found to be random) between homologous chromatids and it has been tacitly assumed that crossing over does not take place between sister strands. There is in fact evidence for the assumption.

STURTEVANT (from results not yet published but soon to appear) has found that crossing over does not take place between sister strands when crossing over is unequal.

The percentages of non-crossovers, crossovers, patroclinous males and inviable eggs in the population from females heterozygous for closed  $X^0$  (table 7) are found to be consistent with expectation if the ends of the closed chromosome are inseparable, and if double or triple complexes resulting from crossing over of chromosomes are lethal, and if crossing over between non-sister chromatids is at random.

If, however, crossing over is random for all four strands the ratios depart from expectation (see table 5). Single, double, and triple exchanges between homologous chromatids would result in equal numbers of closed  $X^0$  and normal X non-crossovers, which is almost certainly the true ratio, there being a relatively greater inviability in the closed  $X^0$  class in stages after egg laying. If sister strands cross over, single exchange between normal sister strands would give rise to viable apparent non-crossovers, but between closed sister chromatids would give rise to an inviable double complex, reducing the percent of closed  $X^0$  non-crossovers and also increasing the percent of inviable eggs or of patroclinous males. Double exchange if it included exchanges between sister strands would also change the ratios in the same direction. The expected ratios due to different kinds of exchange are given in table 5.

The observed ratios then show that sister strand crossing over if it occurs at all is not as frequent as crossing over between homologues. WEINSTEIN (1932) has reached the same conclusion mathematically.

Evidence that sister strand crossing over does not occur when conjugation of homologous chromosomes is prevented is derived from the experiment with heterozygous closed  $X^0$  and inverted X (appendix, experiment J<sub>1</sub>, table 13). Conjugation between homologous strands was almost prevented by the inversion  $\Delta$ -49 and inviable complexes from single and odd numbered exchanges would have lowered the percent of the closed  $X^0$  non-crossover class if exchange had occurred between sister closed  $X^0$  chromatids. But no reduction was observed; the ratio of closed to normal non-crossovers was no more different from 1:1 than the deviation shown by other experiments to be probably due to differential inviability.

#### SUMMARY

A female *Drosophila* carrying attached X chromosomes and homozygous for yellow produced a daughter with a single maternal X chromosome that was "closed" ( $X^0$ ).

The closed  $X^0$  may conjugate with another X chromosome since double crossovers are recovered from females heterozygous or homozygous for closed  $X^0$ .

There is genetic evidence that the chromosome is closed by union of its ends since the loci for yellow and bobbed have never been found to be separated by crossing over, nor have the characteristics of closed  $X^0$  ever been separated by crossing over from either of the two loci, which are near the ends of the chromosome.

No single crossovers are recovered from females carrying closed  $X^0$  whether homozygous or heterozygous.

The percents of double crossovers among the normal offspring of females heterozygous for closed  $X^0$  are somewhat lower than among normal controls for every region of the chromosome. The most marked difference in frequency of doubles is at the left end of the chromosome. This region in the closed chromosome is near the spindle fibre attachment, a condition which is known to decrease crossing over.

In cytological preparations the closed chromosome appears as a slightly elongated closed ring.

Low viability of closed  $X^0$  is shown by the great difficulty in maintaining homozygous stock under the most favorable conditions, by low frequency of closed  $X^0$  males in backcrosses, and by a high percentage of closed  $X^0$  classes among dead pupae from backcrosses. The survival of some adults and the observations on dead pupae indicate a variable degree of inviability after the egg stage.

A single exchange of segments (crossing over at one level only) between a closed  $X^0$  and a normal  $X$  chromatid would result in a double complex, consisting of two chromatids attached together, because the two ends of the closed chromatid are united. Among  $F_1$ s from flies heterozygous for closed  $X^0$  there is no class of non-disjunctional females containing united  $X$  chromosomes as would be expected if double complexes survive.

A 2-strand double exchange (one in which only two homologous chromatids take part) would produce two separate double crossovers, one closed, one normal.

A 3-strand double exchange in which two normal strands and one closed strand take part would produce a normal double crossover.

The other kind of 3-strand double exchange, in which two closed chromatids and one normal chromatid take part, would unite three chromatids into one complex, and no closed  $X^0$  double crossover would be recovered.

A nearly 1:2 ratio of the percent of closed  $X^0$  double crossovers (among the closed  $X^0 F_1$ s from heterozygotes) to the percent of normal  $X$  doubles (among normal  $X F_1$ s) has been observed. It is the ratio to be expected if homologous strands cross at random; that is if 2-strand and each of two kinds of 3-strand double exchange are equal. These results together with the equality of 2-strand and 4-strand double exchange found by EMERSON and BEADLE (in press) in attached  $X$  show that exchange between homologous strands is at random.

Patroclinous males are about  $\frac{1}{3}$  as frequent as normal  $X$  double crossovers from heterozygotes; these might result from irregularities due to formation of united chromatids at maturation and together with inviable eggs represent classes due to exchange.

Patroclinous males would be expected from the second kind of 3-strand double exchange if two united chromatids going to one pole dragged the third one that is also united to them out of its nucleus leaving one nucleus with a single X chromatid after the first division. The expected frequency of patroclinous males from 3-strand exchange would be  $1/4$  of the frequency of normal double crossovers, a little less than the observed  $1/3$ . Some patroclinous males may be derived rarely from other exchanges.

The percent of double crossovers from homozygotes is in agreement with the conclusions reached from heterozygotes in regard to double crossovers. The true percent of patroclinous males from homozygotes is obscured by low viability of regular classes but the observed percent appears to be consistent with expectation on the assumption that patroclinous males are derived mainly from 3-strand double exchange.

Lethal effects of exchange would affect the egg stage of *Drosophila*. In-viable eggs were found to be 33.2 percent of the deduced number representing the total population in  $F_1$  from females heterozygous for closed  $X^0$ . This is in agreement with the hypothesis that patroclinous males are derived mostly from one kind of 3-strand double exchange and that double chromatid complexes resulting from one exchange between two chromatids are lethal. On the hypothesis, patroclinous males, which are about 2.3 percent of the population, and an equal number of YO gametes, which are lethal in the egg stage, represent one kind of double crossover, and the remainder of the lethal eggs, or about 31 percent of the population, represent single crossovers. The percents are in agreement with the hypothesis if crossing over takes place somewhat less frequently between closed  $X^0$  and normal X than between normal Xs. Evidence for reduced frequency of crossing over is found in a relatively high percent of non-crossovers among  $F_1$ s from flies heterozygous for closed  $X^0$ , and a relatively low percent of double crossovers from heterozygotes as compared with values obtained from controls.

If the first meiotic division when closed  $X^0$  is present is reductional, nuclear division after exchanges that produce double complexes would be expected to lead to severed chromatids, but no flies containing corresponding deficiencies or duplications have been observed. Patroclinous males would be expected if some nuclei were formed without any X chromatid.

If the first meiotic division is the kind of equational division that would segregate two single chromatids from a double lethal complex the observed ratios are most nearly satisfied.

If inviable eggs and patroclinous males result from irregularities of crossing over their frequency should be reduced if crossing over is reduced. Heterozygotes for closed  $X^0$  and normal X gave 31 percent of inviable eggs; when crossing over was reduced by an inversion in an X chromosome,



heterozygotes for closed  $X^0$  and the inversion ( $\Delta$ -49) gave only 6.3 percent of inviable eggs.

Double crossing over is almost entirely prevented and single crossing over is reduced to 13 percent in heterozygotes for normal X and inversion  $\Delta$ -49. Heterozygotes for closed  $X^0$  and  $\Delta$ -49 gave 6.3 percent of inviable eggs but no excess of patroclinous males over the percent obtained from heterozygotes for  $\Delta$ -49 and normal X. This agrees with the hypothesis that with closed  $X^0$ , patroclinous males represent one kind of double crossover, and inviable eggs represent almost entirely single crossovers, crossing over being relatively infrequent when closed  $X^0$  is present.

The observed ratios are those to be expected if exchange between homologous strands is random and if sister strands do not cross over.

When conjugation between homologous strands is almost prevented by an inversion in the apposing X chromosome of a female heterozygous for closed  $X^0$ , the ratio of the two classes of non-crossovers in  $F_1$  is 1:1 which is not expected if sister strands cross over independently of crossing over between homologues.

The conclusion that homologous strands cross at random applied to unpublished data of BRIDGES from completely marked X chromosomes, indicates that exchange between normal X chromosomes probably takes place as a rule in all eggs of *Drosophila melanogaster*.

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APPENDIX  
TABLES 10-13

For every type of complementary offspring the class first recorded is that containing the left end of the maternal chromosome that is described above the line.

TABLE 10  
*Experiment A<sub>1</sub> and A<sub>2</sub>;*  
♀ offspring of  $\frac{X^b y \quad cv \quad v \quad f}{ec \quad c^b \quad g^2 \quad bb}$  ♀ ×  $y \quad ec \quad cv \quad c^b \quad v \quad g^2 \quad f \quad bb(X_A) \sigma^7$ .  
1 2 3 4 5 6 7

*Experiment A<sub>2</sub>;*  
offspring of  $\frac{X^b y \quad ec \quad c^b \quad g^2}{sc \quad br \quad w^a \quad cv \quad v \quad f}$  ♀ ×  $sc \quad w^a \quad ec \quad cv \quad c^b \quad v \quad g^2 \quad f \sigma^7$ .  
1' 1'' 1''' 2 3 4 5 6 7

*Experiment B<sub>1</sub>;*  
offspring of  $\frac{X^b y \quad cv \quad v \quad f}{sc \quad ec \quad c^b \quad g^2 \quad Bx^2 \quad bb}$  ♀ ×  $X_A \sigma^7$ .  
1 2 3 4 5 6 7' 7''

*Experiment B<sub>2</sub>;*  
offspring of same type of ♀ ×  $y \quad ec \quad cv \quad c^b \quad v \quad g^2 \quad f (X_7) \sigma^7$ .

EXPERIMENT	TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED	
A <sub>1</sub> , A <sub>2</sub>	0	1849	2070	1,6	5	19	2,6	11	44
A <sub>a</sub>		1687	3298		12	35		12	64
B <sub>1</sub>		1333	1600		5	14		6	32
B <sub>2</sub>		1371	1495		6	10		5	25
Total	0	6240	8463	1,6	28	78	2,6	34	165
A <sub>1</sub> , A <sub>2</sub>	1,2	2	0	1,7	3	18	2,7	10	37
A <sub>a</sub>		1	3		4	31	.	14	63
B <sub>1</sub>		0	1		2	12		5	23
B <sub>2</sub>		0	0		1	12		8	9
Total	1,2	3	4	1,7	10	73	2,7	37	132
A <sub>1</sub> , A <sub>2</sub>	1,3	1	1	2,3	0	0	3,4	6	7
A <sub>a</sub>		0	0		0	1		5	4
B <sub>1</sub>		0	0		0	1		1	8
B <sub>2</sub>		0	0		1	0		0	8
Total	1,3	1	1	2,3	1	2	3,4	12	27
A <sub>1</sub> , A <sub>2</sub>	1,4	6	8	2,4	3	13	3,5	6	18
A <sub>a</sub>		7	9		7	18		10	34
B <sub>1</sub>		1	6		3	11		3	16
B <sub>2</sub>		1	2		5	8		5	8
Total	1,4	15	25	2,4	18	50	3,5	24	76

[illegible]

TABLE 11  
*Control of Experiment A<sub>1</sub> and A<sub>2</sub>. Female offspring of*

$\frac{sc}{(sc)} \frac{cv}{ec} \frac{v}{c^a} \frac{f}{g^a} \frac{bb}{bb} \quad \varphi \times X_8 \sigma^7$ . For each type, the second class contains *ec*.

TYPE		NUMBER OBSERVED		TYPE		NUMBER OBSERVED		TYPE		NUMBER OBSERVED	
0	558	557	2,6	12	22	5,7	11	8			
2	71	94	2,7	19	12	6,7	5	2			
3	65	80	3,4	2	3	2,3,7		1			
4	162	150	3,5	10	5	2,4,6	1				
5	107	99	3,6	14	14	2,4,7	1	1			
6	104	111	3,7	11	9	2,5,7	1	2			
7	63	97	4,5	5	11	3,5,7		1			
2,3	2	1	4,6	22	22	4,5,7		1			
2,4	4	9	4,7	17	13	4,6,7		1			
2,5	15	11	5,6	1	2	3,4,5,7		1			
Total non-crossover		1115	Total single and triple		1213	Total double and quadruple		295			
Grand total 2623											

TABLE 12  
*Experiment D<sub>a</sub>;*  
 $\sigma^7$  offspring of  $\frac{X^{0y}}{X^{0y}} \frac{cv}{ec} \frac{v}{c^a} \frac{f}{g^a} \quad \varphi \times bb \text{ Y } \sigma^7$ .  
1 2 3 4 5 6 7  
*Experiment D<sub>b</sub>;*  
 $\sigma^7$  and  $\varphi$  offspring of  $\frac{X^{0y}}{X^{0y}} \frac{cv}{ec} \frac{v}{c^a} \frac{f}{g^a} \quad \varphi \times X_8 \sigma^7$  or  $X_7 \sigma^7$ .  
1 2 3 4 5 6 7

EXPERIMENT	APPARENT TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED	
D <sub>a</sub>	0	48	46	2,3	0	0	3,6	0	0
D <sub>b</sub>		1336	1032		1	0		7	7
Total	0	2462			1			14	
D <sub>a</sub>	2	0	2	2,4	1	0	4,5	0	0
D <sub>b</sub>		4	6		2	6		3	7
Total	2	12			9			10	
D <sub>a</sub>	3	0	0	2,5	0	1	4,6	1	0
D <sub>b</sub>		6	8		9	4		8	5
Total	3	14			14			14	

TABLE 12 (Continued).

EXPERIMENT	APPARENT TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED		TYPE	NUMBER OBSERVED	
D <sub>a</sub>	4	0	0	2,6	0	1	5,6	1	0
D <sub>b</sub>		11	14		4	6		4	6
Total	4	25			11			11	
D <sub>a</sub>	5	0	0	3,4	0	0	non-disjunc- tional ♀♀		
D <sub>b</sub>		10	11		4	1		2	
Total	5	21			5				
D <sub>a</sub>	6	0	0	3,5	0	0	gynandro- morphs		
D <sub>b</sub>		9	5		4	5		1	
Total	6	14			9				

*Values deduced from apparent type 0 and apparent singles*

TYPE NUMBER	0	1,2	1,3	1,4	1,5	1,6	1,7	2,7	3,7	4,7	5,7	6,7
	2457	1	1	6	8	9	5	11	13	19	13	5
Total	regular classes	2646		non-cross-overs		2457		double crossovers		189		

*F<sub>1</sub>'s of females mated to X<sub>1</sub> males*

Total	2121	Patroclinous ♂♂	254	Per-cent	12.0
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TABLE 13

*Classes observed in experiments in which eggs were counted on the first day and on the fourth day after laying.*

	NON-CROSSEOVERS		CROSSEOVERS		PATROCLINOUS ♂♂	EGGS TOTAL INVIALBE	NON-DISJUNCTIONAL ♀♀
J <sub>1</sub> X <sup>o</sup> y/Δ-49 cm <sup>±</sup> bb × y Δ-49 cm bb first 6 days	y	cm	y cm	+	y Δ-49 cm bb		+
	2317	3157	0	0	inviable	3383 217	1
J <sub>2</sub> X <sup>o</sup> y/g <sup>a</sup> × yg bb first 6 days	y	g <sup>a</sup>	yg <sup>a</sup>	+	yg <sup>a</sup> bb		+
	1302	1535	136	393	135		among
	754	849	73	224	74	3326 1040	crossovers
J <sub>3</sub> g <sup>a</sup> /Δ-49 × g <sup>a</sup> first 6 days						3498 112	

# X-RAY EFFECTS ON DROSOPHILA PSEUDO-OBSCURA

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In order to procure new material for the study of problems connected with the non-disjunction of the sex chromosomes in *Drosophila pseudo-obscura*, a series of X-ray experiments was carried out. Among the species of *Drosophila*, *pseudo-obscura* is distinguished by the length of its X chromosome, whose genetic map is the longest recorded in any species (LANCEFIELD 1922). The work of LANCEFIELD has made a number of mutants available for experiments with this chromosome. Accordingly, the experiments were arranged to permit the detection of (1) non-disjunction of the sex chromosomes in the treated females, (2) crossover modifications in the treated X chromosomes, and (3) non-disjunction of the X chromosomes in the progeny of treated individuals. An eosin ( $w^e$ ; locus 68) garnet-2 ( $g^2$ ; locus 88) stock was irradiated, and the treated females were mated to wild type males. In the progeny, exceptional individuals, resulting from non-disjunction of the sex chromosomes in the mother, could be detected. The regular  $F_1$  females were mated to males carrying in their X chromosome the dominant gene Pointed; among their offspring, the sons gave the measure of crossing over in the eosin garnet-2 interval, and the presence of the dominant sex-linked gene in the father permitted the detection of non-disjunction of the X chromosomes in the mother.

The reciprocal cross was also carried out: eosin garnet-2 males were treated and mated to wild type females. The daughters in this cross were of the same constitution as those obtained from the reciprocal mating, and were tested in the same manner. In these tests of  $F_1$  daughters, the untreated chromosome may be considered to serve as a control. There is no control series available for the mating of irradiated female to normal male; for the reciprocal cross, however, a control was raised.

The X-ray dosage was the usual one of MULLER (1927); the Coolidge tube used at a peak voltage of 50 K.V., with a 5 m. am. current, the flies at a distance of 16 cm. from the target, with a 1 mm. aluminium filter. The exposure was sixty minutes for the females and for one set of males, but another set of males was treated for seventy-five minutes.

These experiments were carried out in the winter of 1929 and the spring of 1930, and have been reported briefly in the Carnegie Year Book for 1930 (MORGAN, BRIDGES and SCHULTZ 1930).

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## THE DATA

The most striking effects were observed in the progeny of the irradiated males that were mated to normal females. There was a marked deficiency of females in the progeny from the experimental cultures, although the sex ratio in the controls was 1.16 ♀ to 1.00 ♂. This deficiency of females was most marked, as might be expected, in the series given the heaviest treatment (table 1). In that case 66 percent of the expected number of females were lacking.

TABLE 1

*Progenies of treated males + ♀ ♀ × w<sup>8</sup> g<sup>8</sup> ♂♂, ♂♂ exposed to X-rays; 50 K.V., 5 m. am., 1 mm. A1 filter distance 16 cm.*

EXPOSURE MINUTES	NUMBER OF CULTURES	♀ ♀	♂♂	SEX RATIO	SEX RATIO (EXP)	PERCENT ♀ ♀ DEFICIENT
					SEX RATIO (CONTROL)	
60	26	349	634	0.55	0.47	0.53
75	30	185	435	0.40	0.34	0.66
0	8	554	475	1.16	..	..

There was also a high percentage of abnormalities in the progeny; 3.5 percent of the total in the sixty minute treatment, and 2.7 percent in the seventy-five minute treatment. The two largest groups consisted of mutants of the Minute type, with the usual small bristles and other characteristic peculiarities of this class (BRIDGES and MORGAN 1923, SCHULTZ 1929), and of a number of males which had no gonads but were otherwise normal (table 2). These were very similar to the "castrated" males described by GEIGY (1927, 1931) as resulting from treatment of eggs with ultra violet light. In *Drosophila pseudo-obscura*, the absence of testes is particularly striking, since the envelope of the gonad is colored a bright orange, and is easily visible through the abdominal wall. In these abnormal males the abdomen appeared white. On dissection it became apparent that the testes were absent, but the rest of the reproductive system was normal.

TABLE 2

*Abnormalities occurring as a result of treatment.*

EXPERIMENT	TOTAL ABNORMAL IN F <sub>1</sub>	♂♂ WITHOUT TESTES	STERILE MUTANTS	F <sub>1</sub> MUTANTS RETAINED	F <sub>1</sub> ♀ ♀ TESTED FERTILE CULTURES	STERILE CULTURES	VISIBLE SEX LINKED RECESSIVES	LETHALS
60 min ♂♂	35	13	10	7	46	11	6	8
75 min ♂♂	17	1	6	2	5	7	1	..
60 min ♀ ♀	12	..	4	..	45	23	3	4
Control	..	..	..	..	..	..	..	..



In addition to these two groups there appeared such characters as "Star" eyes, "Plexate" venation of the wings, "Spread" wings, "Confluent" wing veins, and "Delta" venation. Many of these proved sterile, but some were fertile and stocks of these were established.

The effects observed in the progeny of the treated females were much less extreme. There was in  $F_1$  a slight deficiency of males, somewhat more than might be expected as a result of the poorer viability of the eosin garnet-2 males as compared with their wild type sisters. In the absence of a control series, the rather wide fluctuations of the sex ratio in *Drosophila pseudo-obscura* in different experiments make it difficult to place much weight on this result. The occurrence of sex linked lethals in the mother would lead to such a deficiency of males and would thus be expected in progenies from X-rayed mothers.

TABLE 3  
Progenies of treated females  $w^a g^2 \text{♀} \times + \text{♂} \text{♂}$ ,  $\text{♀} \text{♀}$  treated as the  $\text{♂} \text{♂}$  in table 1.

EXPOSURE (MINUTES)	NUMBER OF CULTURES	♀ ♀	♂ ♂	SEX RATIO
60	37	887	547	1.60

The number of abnormal specimens<sup>2</sup> was much lower in this group than in the progeny of treated males, a fact which gains significance when it is remembered that new sex-linked recessives in the treated chromosomes may be detected in this cross, which is not the case when males are treated. In spite of this, only 0.8 percent of the total progeny were variants. Of these, one was an occurrence of Notch—probably an oogonial mutation, since three such females, all of which were sterile, appeared in one culture. There occurred also a singed male (sterile), and several mosaics, which may have been due to fractional mutation<sup>3</sup>; a male with one wing curled, another with one miniature wing, and a third male with the venation plexus on one wing.

It is clear that the effects in  $F_1$  are much more extreme when the males are treated. This is further evidenced in the tests of  $F_1$  daughters. Here again (the last four columns of table 2) the males treated for sixty minutes are much harder hit than the corresponding females.

Those of the mutants that could be carried on were studied. It developed that a number of them carried translocations, indicating as might be ex-

<sup>2</sup> Only one male resulting from primary nondisjunction in the treated females was found.

<sup>3</sup> These were not tested. Their occurrence is of interest in connection with the work of PATTERSON (this journal, 1933, pp. 32-52) on *D. melanogaster*, where mosaics of this kind do not occur in the female. Among the progenies of X-rayed males, five "fractional" mutations were found among the 52 aberrants of the present experiment. These also were not tested; further data are necessary for the study of this problem.

pected, that these occur frequently following the radiation of sperm in this species. Six chromosome aberrations were found: three translocations, one involving the second and third autosomes, the others the second chromosome and the Y; a high non-disjunction stock, probably a translocation, since in such cultures sterile males, probably carrying duplications for a part of the X, appeared; and finally, two crossover reducers, one inhibiting crossing over between eosin and garnet-2, the other between yellow and eosin. The sex-linked recessives which appeared included yellow, a prune-like eye color located some twenty units to the left of yellow, an extreme short vein character associated with a total loss of the testes, an echinus-like eye, a rudimentary-like wing, and a "bubble" wing which proved allelomorphic to the similar mutant found by LANCEFIELD (1930) in race B.

#### DISCUSSION

The deficiency of females in progenies from X-rayed males is a phenomenon discovered by MULLER in his experiments of 1927 on *Drosophila melanogaster*. He offered the explanation that "dominant lethals" occurred in the X chromosomes as a result of irradiation, and that females heterozygous for an X chromosome carrying such a lethal died. Since the Y chromosome is genetically almost empty, the males in the progeny have an advantage over the females who receive from their fathers a possibly injurious X instead of the innocuous Y. This manifests itself in the changed sex ratio.

In MULLER's data on *D. melanogaster*, the maximum effect found was an 18 percent deficiency of females, and this of doubtful statistical significance. The present data on *D. pseudo-obscura* show a much greater depression of the sex ratio, the deficiency of females reaching sixty-six percent of the number to be expected on the basis of the control values. It will be remembered that the X chromosome of *D. pseudo-obscura* is very long; in fact, it contains about forty-three percent of the haploid chromatin, as contrasted with twenty-eight percent in the *melanogaster* X chromosome. The more extreme results with *D. pseudo-obscura* are then to be expected on MULLER's hypothesis, since the longer the X chromosome, the greater the chance that a "dominant lethal" will arise, hence the greater the depression of the sex ratio. The problem is complicated, however, by such factors as the comparative extent of the "inert region" of the X in the two species, and the relative importance of different regions and different chromosomes in the production of dominant lethals. It is entirely likely that these represent long deficiencies, or translocations resulting in chromosomes with two spindle fibres, which would be lethal.

In this connection, MULLER's attempt to test the assumption that "dominant lethals" occur at random throughout the chromosomes may be

mentioned. On this assumption he deduced a relation between the depression of the sex ratio, the percentage of eggs laid that hatched into adult females and the proportion of the total haploid chromatin contained by the X chromosome. He found this relation not to hold in his data, nor does it hold in HANSON'S (1928) data even when correction is made for the "inert" region (PAINTER 1931, MULLER and PAINTER 1932, DOBZHANSKY 1932) of the X. Many more eggs die than should according to formula. MULLER therefore considered that some type of effect on more than one chromosome might be responsible. The explanation of the discrepancy, offered by EFROIMSON (1931), that an autosomal deficiency of a given length is more likely to be a dominant lethal than one in the X chromosome, on considerations of genic balance, seems unlikely. For example, a relatively small section of the X is involved in PATTERSON'S "viability gene" (1932) which behaves as a dominant lethal under his experimental conditions, although BURKART (1931) appears to have obtained from the "Blond" translocation a deficiency for this section which survives as a Minute. Furthermore deficiencies in the autosomes, involving several units of the map, are known to be viable. It seems preferable to adopt the view that the many possible types of multiple chromosome aberrations resulting from X radiation are responsible for the excess mortality.

The depression of the sex ratio seems to be the easiest way of measuring the gross X-ray effects in those experiments where the interest is centered on the magnitude of the effect rather than on the detailed analysis of the various types of disturbance. In this respect, *D. pseudo-obscura* is an especially favorable object due to the extent of the effect. Yet by matings of the treated males to attached X females in *D. melanogaster*, a converse deficiency of males may be obtained (BARTH 1928); this is, of course, due to the dominant lethals plus the sex linked recessive lethals. These two quantities combined give a discrepancy of sufficient magnitude to be workable in *D. melanogaster*. The technique has the advantage of being less laborious than the usual ClB method of MULLER, and therefore may have a certain use in the further analysis of the effects of X-rays on chromosomes, and particularly in exploratory work with other agents.

It may be remarked in the present work, as in that with *D. melanogaster*, that the X-ray effect is noticeably less upon the female than upon the male (MULLER 1930, PATTERSON and MULLER 1931). In *D. melanogaster* this has also been shown to be the case for the immature germ-cells of the male as compared with the mature sperm (HARRIS 1929, HANSON 1929) suggesting that the difference in effect may be perhaps correlated with the difference between haploid and diploid. It has been indicated that the difference between the effect on male and female in *D. melanogaster* is due largely to a difference in the number of chromosome abnormalities (PAR-

TERSON and MULLER 1931). It is possible that the usual number of these abnormalities may be produced in the diploid cell, but that they are eliminated because a greater proportion of types, which cannot undergo division normally, are produced. It should also be remembered however, that the relative distances between chromosomes are greater in the immature cells than they are in the sperm, hence translocation might less frequently be successful. When these considerations are taken into account, the observed differences are perhaps to be expected.

The males without gonads found in  $F_1$  from the treated males deserve special mention. Their condition is not due to loss of the Y chromosome, since XO males of this species are normal in testis size. The lack of testes must then be either a developmental abnormality caused by X-rays, or a rather frequent kind of dominant mutation, affecting either the initial establishment of the germ track, or the later development of the gonad. It should be recalled here that among the mutants induced by X-rays, one sex-linked recessive caused a loss of the testes, associated with a short vein character in the wings. The male sterility observed by MULLER in his experiments with *melanogaster* may be due to the same phenomenon, which is less easily observed in *D. melanogaster*, since the color of the testis envelope is relatively inconspicuous.

An accurate comparison of the effects of X-rays on *D. melanogaster* and *D. pseudo-obscura* cannot be made at present since no strictly comparable data are available. The comparison might be interesting, in view of the results of TIMOFÉEFF-RESSOVSKY (1931) on *D. funebris*, where the data, also unfortunately not strictly comparable, indicate a lower lethal mutation rate than in *melanogaster*. In *D. pseudo-obscura* it is the impression that dominant mutations are more frequent than in *D. melanogaster*, both spontaneous mutations and those from X-rays.

#### SUMMARY

1. X-ray experiments were made with *Drosophila pseudo-obscura*, which were so arranged that non-disjunction could be detected in the treated females, and modifications of crossing over and disjunction of the X chromosomes could be detected in the  $F_1$  both from treated males and females.

2. In the progeny of treated males, instead of the sex ratio of 1.16 ♀ : 1.00 ♂ observed in the controls, there occurred a marked decrease from the expected number of females. Further a high percentage of abnormalities was found. They consisted mostly of Minutes and males without gonads, and a number of other mutants in addition. Six chromosome aberrations were found as a result of treatment.

3. As has been found in the work with *D. melanogaster*, the effects noted in the progeny from treated females were less extreme than in those from treated males.

4. The more extensive depression of the sex ratio observed in *D. pseudo-obscura* than in *D. melanogaster*, in the progenies from treated males, may be correlated with the difference between the relative sizes of X and autosomes in the two species. The longer the X relative to the autosomes, the greater the chance of injury to the females carrying such chromosomes that have been irradiated.

5. The depression of the sex ratio in *D. pseudo-obscura* affords the simplest technique available for measuring gross X-ray effects on chromosomes. A similar technique in *D. melanogaster* is available from the mating of treated males to attached X females.

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# THE GENETICS AND CYTOLOGY OF A TETRAPLOID FROM *OENOTHERA FRANCISCANA* BARTLETT<sup>1</sup>

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Tetraploid plants of *Oenothera* have appeared in a number of lines under investigation and from unrelated material. The first plant, *gigas* from *Lamarckiana*, was discovered by DE VRIES (1901) in 1895 and he records that *gigas* also arose from seed of *sublinearis* in 1898 and from the cross *lata* × *hirtella* in 1899 although the latter of these two plants did not mature. MACDOUGAL (1907) reports the appearance of one plant of *gigas* from his cultures of *Lamarckiana* and SCHOUTEN (1908) lists three plants from commercial seed of *Lamarckiana*. HUNGER (1913) from large cultures of *Lamarckiana* grown in Java records five plants of *gigas*. HERIBERT-NILSSON (1909, 1912) described a plant from Swedish *Lamarckiana* with characters chiefly those of *gigas*, and GATES (1915) determined its tetraploid chromosome count. GATES (1913) identified as *Lamarckiana gigas* a line from the botanic garden of Palermo and showed it to be tetraploid. A dwarf, *gigas nanella*, from the original line of *gigas* has been studied by DE VRIES (1901, 1915), and SCHOUTEN (1908) obtained a number of these plants. BOEDIJN (1920) established a dwarf tetraploid from *Lamarckiana* mut. *simplex* of DE VRIES. Making allowance for the fact that chromosome counts were not made on some of the plants early reported as *gigas* there are still substantial records of the appearance of this tetraploid from *Lamarckiana* through a number of different lines.

Tetraploids out of species of *Oenothera* other than *Lamarckiana* or its derivatives have been recorded from several sources, the most important of which are the following: BARTLETT (1915a, b) reported *gigas* from *Oenothera stenomerus* and *O. pratincola*. STOMPS (1916) reported tetraploids from the cross *Lamarckiana gigas* × *atrovirens*. A line of *grandiflora* gave tetraploid plants to DE VRIES (1918) and to BOEDIJN (1924). STOMPS (1925) obtained from a cross between *biennis* and *biennis cruciata*, a *biennis gigas*, and crosses of this tetraploid with *biennis cruciata* gave *biennis cruciata gigas*. Plants of *biennis cruciata gigas sulfurea* were shown by STOMPS in the exhibition garden of the Sixth International Congress of Genetics at Ithaca in 1932. There has also been recorded the appearance of tetraploids from crosses of *gigas* and *semigigas* with certain hybrids (BOEDIJN 1924). To this list may now be added a tetraploid by way of my line of *Oenothera franciscana* B, a species with all pairing chromosomes.

<sup>1</sup> Cytological Studies on *Oenothera* V. Papers from the Department of Botany, UNIVERSITY OF MICHIGAN, No. 351.



With respect to the origin of the tetraploid *Oenotheras* there have been two principal and opposing views. GATES (1909a) held that "the double number of chromosomes in *O. gigas* originated soon after fertilization by the failure of a nucleus to complete its division after the chromosomes had divided, a condition comparable to the monasters of BOVERI," and this interpretation was supported by STRASBURGER (1910). This view was soon criticized by STOMPS (1912a, b) and by LUTZ (1912) and has not been favorably received by DE VRIES (1913) or generally by other workers on *Oenothera* cytology and genetics who believe with STOMPS that diploid gametes are developed and function. These diploid gametes may rarely meet to produce the tetraploid; more frequently they mate with normal gametes to give the well known triploid *Oenotheras* out of which tetraploids may come. The origin of my tetraploid from *franciscana* was by way of a triploid thus supporting the conclusions of STOMPS.

Triploid *Oenotheras* derived from *Lamarckiana* were first established through chromosome counts by STOMPS (1912a) and LUTZ (1912). STOMPS wrote of them under DARWIN's term of *hero* plants and gave them the name *semigigas* and these names are found in many papers on *Oenothera*. Triploids may be expected to produce a much larger proportion of diploid gametes than do diploids and the chances of an origin of tetraploids by way of triploids are correspondingly greater than the possibilities of an origin from diploids direct. Recent cytological work on haploid *Oenotheras* by DAVIS and KULKARNI (1930), EMERSON (1929) and STOMPS (1930) has shown the manner by which meiosis may be eliminated from the history of pollen formation in *Oenothera*, and the early observation of GEERTS (1909, p. 52) of an embryo sac in *Lamarckiana* with 28 chromosomes appears to have been a case of similar suppression of meiosis in the ovule. Thus the failure of a heterotypic spindle to develop or to function with the consequent reconstitution of a nucleus which then passes through the homoeotypic division would give two diploid spores. Or, the suppression of the homoeotypic division following a normal heterotypic division, but after the lengthwise division of the chromosomes, would produce a diploid spore. Both of these irregularities give the same diploid chromosome set when they occur in homozygous material, but in heterozygous material spores from suppressed homoeotypic divisions would express such chromosome peculiarities as might result from segregation in the heterotypic division.

With respect to the possibility of the origin of triploids through dispermy we have the interesting report and figure of ISHIKAWA (1918) for *Oenothera nutans*  $\times$  *O. pycnocarpa*, and the observations of NEMEC (1912) on *Gagea*. No evidence for dispermy was reported through the careful work of GEERTS (1909) and the studies of RENNER (1914) and others on the

embryo sac of *O. Lamarckiana* and other species. Neither is there known any genetical behavior of *Oenothera* triploids that would suggest an origin through dispermy. Since the investigations on dispermy in animal material have established irregularities of subsequent chromosome distribution and the failure of the embryo to attain maturity, usually with its early death, we may scarcely hope that dispermy will prove to be responsible for the vigorous triploids produced by plants. The rapidly increasing reports of the production of diploid gametes in plants through the suppression of the first or second divisions of meiosis offer the only evidence on which we are at present justified in drawing conclusions. GATES (1928) has, however, expressed the view that triploids have almost certainly arisen through dispermy.

There is apparently no *Oenothera* material known that supports the interesting views of WINGE (1917, 1932) on the origin of constant species-hybrids as tetraploids resulting from the doubling of the chromosome sets in the  $F_1$  individual. The tetraploids of *Oenothera* which have come directly from diploids have behaved genetically, as far as reported, out of line with WINGE's hypothesis and it seems more probable that they have arisen through the union of diploid gametes. This matter will be considered in the Discussion.

The present study offers the first pedigree of a tetraploid *Oenothera* from a diploid by way of a triploid plant with cytological observations upon the plants directly concerned.

#### METHODS

Except for the  $F_1$  generation from *franciscana*  $\times$  *franciscana sulfurea nana*, grown at the JOHN INNES HORTICULTURAL INSTITUTION, all of the cultures were from seeds forced to germination over wet filter paper in Petri dishes following about 24 hours of alternate exhaust and pressure while soaking in vials of water. This treatment very much hastens germination apparently through the removal of air from the pores of the seed coats and the forcing of water against the contents of the seeds. Generally almost all of the viable seeds have germinated within 10 days and the remainder usually germinate during the next week. Seedlings are removed as they germinate to other Petri dishes and the residue in the first dish constitutes the mass of sterile seeds. When large enough to handle easily with the forceps the seedlings are planted in flats. There is little mortality among the seedlings, those that die being generally weaklings that are unable to free themselves without assistance from the seed coats. I greatly appreciate the kindness of Prof. BATESON who in 1925 gave space in the gardens at Merton for a set of cultures upon which I made observations and selfings.

It is fortunate that cytological material was collected from the triploid and tetraploid of this line so that the origin of the tetraploid is known from both the genetical and cytological side. I am indebted to Dr. ELLIOT WEIER for the material of the triploid (26.51-477) which was fixed in Strong Flemming and in Zenker's fluid, and for the material of the first tetraploid (27.36-18) fixed in Allen's modification of Bouin. The material of the second tetraploid (28.34-55), collected by Dr. KULKARNI, was fixed in a modification of Bouin somewhat stronger in chromic acid and urea and proved particularly good (Bouin's fluid 100 cc, 1 g chromic acid, 2 g urea).

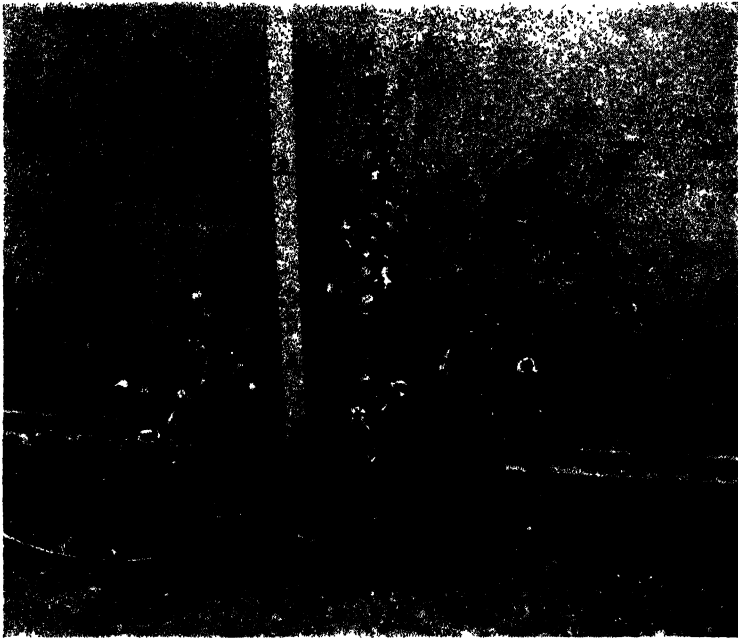
Iron alum haematoxylin gave its usual dependable results but the best preparations were stained by a modification of the Gram method as recommended by NEWTON. The procedure for this material was as follows: (1) Stain for 20 minutes in a solution of crystal violet or gentian violet, 1 g in 100 cc water. (2) Rinse in water and then for 30 seconds move the slide about in a solution of 1 g iodine and 1 g potassium iodide in 100 cc 80 percent alcohol. (3) Carry slide very rapidly through alcohols to clove oil. (4) Remove clove oil with xylol. The differentiation of the stain takes place chiefly in clove oil. If it proceeds too rapidly hasten the dehydration and for clearing use a mixture of one-third clove oil and two-thirds xylol. If destaining is too slow alternate clove oil with absolute alcohol. Chromosomes should be violet and the cytoplasm almost colorless. The chief advantage of this stain over haematoxylin lies in the translucence of the chromatic material making it possible to focus into lower levels of crowded structures.

#### GENETICS OF THE TRIPLOID AND TETRAPLOID

For a number of years I have been gathering data on the inheritance of three characters of *Oenothera franciscana* Bartlett in contrast with their absence in a series of derivatives from an original cross between *franciscana* and *O. biennis* L. These characters are (1) petal color whether yellow or sulfur (a cream color), (2) stature whether tall or dwarf, and (3) color of bud cones whether with red or wholly green. In the course of this work there appeared a segregate which is sulfur in flower color and dwarf in stature, in contrast to *franciscana* which has yellow flowers and is tall; both forms have bud cones with red, the gene for which is linked with that for stature. The derivative will be called *franciscana sulfurea nana* and it differs from *franciscana* in the absence of one gene that produces yellow flowers and another gene that gives tall stature. I believe that each of these characters, color and stature, depends on the presence or absence of a single gene, but certain peculiar ratios of segregation from crosses have led to experiments not yet completed in an attempt to account for marked deficiencies of sulfur and some deficiencies of dwarfs among the segregates.

The genes for color and for stature are in different chromosomes since they segregate independently from all crosses in which they are brought together.

It is necessary to make this explanation because the triploid, established by cytological study, which produced the tetraploid was one of four similar "hero" plants in an  $F_2$  generation from the cross *franciscana*  $\times$  *franciscana sulfurea nana*. These hero plants (probably all triploids) have appeared in the progeny of a number of different crosses and also once in the selfed line of *franciscana* (culture 24.21a). The triploid then is not a very rare sport.



TEXT FIGURE 1.—*Oenothera franciscana gigas*, a tetraploid arising by way of a triploid selfed, the latter from the  $F_2$  of the cross *franciscana*  $\times$  *franciscana sulfurea nana*.

Both *franciscana* and *franciscana sulfurea nana* are homozygous with sets of 14 pairing chromosomes as shown for the first plant by studies of CLELAND (1922) and KULKARNI (1928), and for the second plant by a study of EMERSON (1928), observations which have several times been confirmed in our laboratory. These chromosomes segregate freely in meiosis. The pollen of both plants is 99 percent good and the seeds are about 90 percent viable.

With respect to the characters under consideration the genetic formula for *franciscana* may be expressed as  $YYTT$  and for *franciscana sulfurea nana*  $yytt$ ;  $Y$  stands for yellow and  $T$  for tall,  $y$  for sulfur and  $t$  for dwarf.

The cross with which the line started was *franciscana*  $\times$  *franciscana sulfurea nana*, and the  $F_1$  generation,  $YyTt$ , was grown at the JOHN INNES HORTICULTURAL INSTITUTION in the summer of 1925. This culture matured 199 plants uniform and *franciscana*-like although a little taller and with somewhat larger leaves and flowers, the latter a slightly deeper shade of yellow perhaps due to thicker petals. The pollen of these  $F_1$  plants was 99 percent good, and the seeds 95 percent viable.

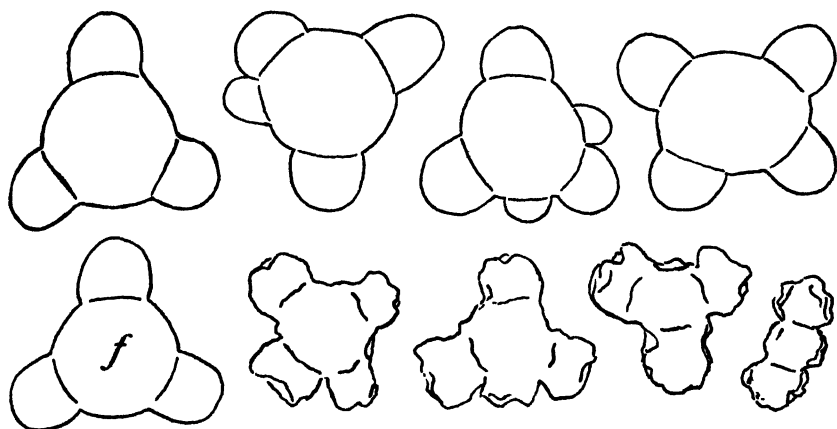
The  $F_2$  generation from seeds of a selfed  $F_1$  plant, ( $YyTt$ ), germination 95.3 percent, consisted of 643 seedlings (culture 26.51, table 1) of which 617 plants were brought to maturity. These were distributed as 498 yellow tall including the 4 hero plants (triploids), 98 yellow dwarf, 16 sulfur tall and 5 sulfur dwarf. The expected segregation, assuming complete fertility of gametes and zygotes, would be 1 sulfur to 3 yellow and 1 dwarf to 3 tall; the actual ratios were 1 sulfur to 28.4 yellow and 1 dwarf to 5 tall, a very marked deficiency of sulfur and a large deficiency of dwarf segregates.

The plant (26.51-477) selected from the four hero types for selfing and which cytological study showed to be a triploid (21 chromosomes) must have arisen from the union of a normal 7 chromosome gamete with a gamete carrying 14 chromosomes. Since the triploid was yellow tall and the tetraploid derived from it was yellow tall and both types threw no segregates for sulfur or dwarf it may be assumed that the 7 chromosome gamete was  $YT$  and the 14 chromosome gamete  $YTYT$ . The  $YT$  gamete, which is the normal gamete of *franciscana*, should constitute one fourth of the gametes developed by the  $F_1$  hybrid  $YyTt$ . The  $YTYT$  gamete could only have arisen through the suppression of the homoeotypic division of a  $YT$  nucleus segregated by the heterotypic division, the 7 chromosomes of which by their lengthwise division previous to interkinesis becoming 14 in number and the nucleus in consequence  $YTYT$ . Fortunately we know that such a suppression of the homoeotypic division through irregularities of spindle development may take place in *Oenothera* for it was observed in the haploid derived from *franciscana* (DAVIS and KULKARNI 1930, p. 73, figure 36).

The triploid plant (26.51-477) was easily distinguished in the field by its stature, taller than *franciscana*, its leaves larger, and by the much thicker bud cones. Only about 30 percent of the pollen in the triploid was well formed of which about half of the grains were 3-lobed and the other half 4-lobed with occasionally a 5-lobed example. The other grains were much shriveled and empty of material, and they also fell into two groups of 3-lobed and 4-lobed grains. Text figure 2 gives some examples of the pollen taken from this triploid plant (26.51-477) and for comparison there is included a pollen grain of *franciscana* (*f*) under the same magnification as the representatives of the triploid. It will be noted that the 4-lobed grains

are larger than those of *franciscana*; they probably carry chromosome counts above the 7 of *franciscana* but not the double set of 14. Pollen grains with 14 chromosomes from the tetraploid (2 sets of *franciscana*) as shown in text figure 3 are very much larger; they are formed very rarely and none chanced to be on the slide preserving the pollen from this triploid plant. Experience has shown that triploids in this material can be recognized with certainty by the peculiarities of the pollen as described above.

The triploid (26.51-477) selfed gave the small harvest of 513 seeds from 11 capsules, less than one-tenth the harvest of *franciscana*, and of these seeds only 116 proved viable, a germination of 22.6 percent. There was heavy mortality among the seedlings and young rosettes such that only



TEXT FIGURE 2.—Samples of pollen from the triploid plant (26.51-477), parent of the tetraploid (27.36-18). Of the 30 percent of good pollen about one-half of the grains were 3-lobed and the other half 4-lobed. A grain of *franciscana* (f) is shown for comparison.  $\times 200$ , reduced one-third.

89 plants lived (culture 27.36, table 1). These were distributed as 3 tetraploids, 42 *franciscana*, 17 dwarfs with narrow leaves and small flowers, and 27 dwarfs of a variety of form that failed to mature.

One of the three tetraploids (27.36-18), which will be called *Oenothera franciscana gigas*, was selected as parent of a tetraploid line and material was collected for the cytological study which established the count of 28 chromosomes. It must have arisen from the union of two 14 chromosome gametes each consisting of two sets of *franciscana* chromosomes. The tetraploid is then to be interpreted as carrying four sets of *franciscana* chromosomes and with respect to the factors for flower color and stature to have the formula *YTYTYTYT*. The 42 *franciscana* plants in the same culture (27.36, table 1) resulted from the union of gametes bearing the single *franciscana* set of 7 chromosomes. These conclusions are based on the findings of the cytological study of the triploid plant (26.51-477), parent of the

culture, in which segregation from the trivalents during meiosis gave occasionally a distribution of 14 but very much more frequently a distribution of 7 chromosomes. The mixed assemblage of dwarfs probably reflected irregularities of chromosome segregation characteristic of the triploid.

The tetraploid, text figure 1, is not quite so tall as *franciscana* but the leaves are much larger and thicker, the bud cones much broader, and the flowers larger. The capsules are shorter and thicker expressing a yield of seeds per capsule less than half that of *franciscana*. In general the tetraploid may be described as more robust than *franciscana* as may be seen by comparing text figure 1 with the published figure of *franciscana* (DAVIS and KULKARNI 1930, p. 57). The pollen is about 20 percent well formed and these grains have 4 or more lobes; very rarely a 3-lobed grain is produced. As shown in text figure 3 the good grains are generally larger than those of the triploid, text figure 2, and very much larger than the pollen grain of *franciscana* (f). The remainder of the pollen consists of shriveled empty grains of various sizes, mostly 4-lobed. As in the case of the triploid, experience has shown that the tetraploid may be recognized with certainty by the peculiarities of its pollen so that an examination of the pollen will separate tetraploids from triploids when other distinguishing characters are less clearly evident.

The first tetraploid plant (27.36-18) of the line produced 159 seeds from 5 capsules which gave a progeny of 59 seedlings (culture 28.34), a germination of 35.9 percent. There matured 41 tetraploids uniform and true to the type; 7 dwarfs failed to flower. The pollen of the plant (28.34-55) selfed for the next generation proved to be 10-20 percent good.

The second generation of the tetraploid line (culture 29.47), from 349 seeds out of two capsules, started with 227 seedlings, a germination of 65.3 percent. There matured 218 tetraploids, uniform; one variant, a broad-leaved dwarf, failed to flower. The plant (29.47-113) selfed for the next generation presented pollen 20 percent good.

The third generation of the tetraploid line (culture 30.41) from 210 seeds out of one capsule, began with 146 seedlings, a germination of 69.5 percent. There matured 129 tetraploids, uniform; 6 narrow-leaved dwarfs failed to flower; a single plant, *franciscana*-like, probably came from the parthenogenetic development of an egg. The plant (30.41-90) selfed to continue the line had pollen 20 percent good.

The fourth generation of the tetraploid line (culture 31.51) from 324 seeds out of one capsule started with 92 seedlings, a germination of 28.4 percent. There matured 54 tetraploids and one plant with narrower leaves. The plant (31.51-43) selfed for the next generation had pollen 10 percent good.

TABLE 1  
*Pedigree of the triploid and tetraploid.*

CULTURE	PARENT	PERCENT OF GER- MINATION	SEEDLINGS	MATURE PLANTS	FAILED TO FLOWER	DIED	GOOD POLLEN IN SELFED PLANT	NUMBER OF SEEDS PER CAPSULE ON SELFED PLANT
F <sub>1</sub> generation J.I.H.I. 1925	<i>franciscana</i> × <i>franciscana</i> <i>sulfurea nana</i>			199 <i>franciscana</i> -like	40 <i>franciscana</i> -like		99 percent	About 500
F <sub>2</sub> generation (26.51)	F <sub>1</sub> plant grown at J.I.H.I.	95.3	643	498 Yellow tall, including 4 triploids 98 Yellow dwarf. 16 Sulfur tall 5 Sulfur dwarf 617	3	23	Triploid (26.51-477) 30 percent	Triploid (26.51-477) About 50
From the triploid (27.36)	Triploid 26.51-477	22.6	116	3 Tetraploids 42 <i>franciscana</i> 17 Dwarfs, narrow-leaved, small-flowered 62	27 Dwarfs	27	First tetraploid (27.36-18) 10 percent	First tetraploid (27.36-18) About 32
From first tetraploid (28.34)	Tetraploid 27.36-18	35.9	59	41 Tetraploids	7 Narrow-leaved dwarfs	11	Second tetraploid (28.34-55) 10-20 percent	Second tetraploid (28.34-55) About 175
From second tetraploid (29.47)	Tetraploid 28.34-55	65.3	227	218 Tetraploids	1 Broad-leaved dwarf	8	Third tetraploid (29.47-113) 20 percent	Third tetraploid (29.47-113) 20 percent
From third tetraploid (30.41)	Tetraploid (29.47-113)	69.5	146	129 Tetraploids	1 <i>franciscana</i> -like 6 Narrow-leaved dwarfs	10	Fourth tetraploid (30.41-90) 20 percent	Fourth tetraploid (30.41-90) About 300
From fourth tetraploid (31.51)	Tetraploid 30.41-90	28.4	92	54 Tetraploids 1 Narrower-leaved 55		37	Fifth tetraploid (31.51-43) 10 percent	Fifth tetraploid (31.51-43) About 125



The chief points in this account of the history of the triploid and tetraploid are summarized in table 1. An interesting feature is the marked uniformity of the flowering populations from the tetraploids throughout the four generations. The few backward plants and dwarfs probably resulted from some unusual chromosome distributions, and the high percentages of shriveled pollen undoubtedly expressed in the main the irregularities of meiosis that break up the *franciscana* sets. The relatively small harvests of seed produced mean similar high percentages of sterility among the ovules. The true breeding of this tetraploid with respect to the plants that mature stands in marked contrast to that of *gigas* from *Lamarckiana* which always gives, when completely represented, a highly varied progeny. This contrast in behavior probably rests on the heterozygous constitution of the *gigas* derived from *Lamarckiana* in contrast to the more stable situation in the tetraploid from the homozygous *franciscana*.

#### CYTOLOGY OF THE TRIPLOID

There was not sufficient material of the triploid (26.51-477) to make profitable a study of the earlier prophase of meiosis in the microsporocyte. This account will then begin with the period of the second contraction which, as shown in plate 1, figure 1, presents a thick chromatic thread for the most part closely gathered but with some loops or arms extending outward from the central mass. The appearance suggests that synaptic associations have already taken place as becomes evident when the loops and other portions of the spirem separate as the nucleus passes out of second contraction. Segments of the chromatic thread are then clearly shown as chromosomes, in pairs and in groups of three (figures 2 and 3). Chains of three segments which are to become trivalents are numerous (figure 3), but no nucleus was observed in which the 21 chromosomes were in 7 chains of 3 each. Nevertheless, such must occur since 7 trisomes were found on a few spindles at metaphase of the heterotypic division (figure 5). Generally there is a mixture of pairs, groups of three, and solitary segments.

Further condensation of the segments gives the short chromosomes characteristic of the heterotypic division and these are assembled in association with a multipolar spindle (figure 4). The chromosomes are mostly in trivalents and bivalents which become very clear as the equatorial plate is established. This is as one would expect in a triploid with all pairing chromosomes. Occasionally a spindle carries 7 trivalents as shown in figure 5 where three of the trisomes from a lower focus are drawn at one side. Usually both bivalents and trivalents are present, as in figure 6, which gives 5 trisomes and 3 pairs; the members of one of the pairs must be a chance association of odd chromosomes that failed to mate with their homologues. Sometimes one or more chromosomes lie quite apart appar-

ently having failed to establish associations with others. The three chromosomes of a trivalent may be grouped in one of two ways as shown in figure 5; either (1) there may be a third chromosome attached to one member of a

*ab*

pair as *ab-ba* or (2) in a chain of three the middle chromosome is bent so that the two end chromosomes lie parallel although not in conjugation as *ab ab*. Either way it is evident that two chromosomes will pass to one

*ba*

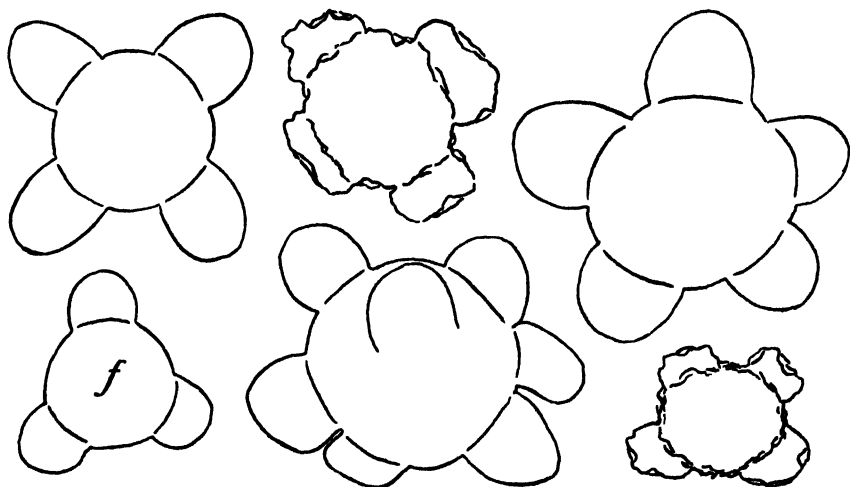
pole and the third to the other pole. When 7 trisomes are present they rarely lie so that the segregation is 7 and 14 although such segregation must take place since nuclei were found at interkinesis with 7 split chromosomes (figure 8), and at the end of the homoeotypic mitosis with 14 chromosomes (figure 16). Usually the trivalents lie with some of the single chromosomes pointing to one pole and some to the other as illustrated in figure 5 where segregation would have given two groups of 9 and 12 chromosomes respectively. Assuming no irregularities of trisome formation and no lagging of chromosomes the mathematical probabilities of a segregation of 7 and 14 chromosomes from a set of 7 trisomes would be 1:128. Thus a very large percentage of the segregation is sure to be irregular even when a full set of trisomes is present.

Other examples of irregularities of segregation are shown in figures 7 and 9 where the distribution of the chromosomes is 10-11 and 8-13 respectively, and figures 10, 11, 12 and 13 give nuclei at interkinesis with counts of 9, 10, 11 and 12 chromosomes. Some of this irregularity results from the fact that very many heterotypic spindles present fewer trisomes than the maximum of 7, the other chromosomes being in pairs (figure 6), or loosely associated. On such spindles the chances of the segregation of sets of 7 and 14 chromosomes is much less than when the full number of trisomes is present.

Interkinesis (figures 8-13) presents the split chromosomes characteristic of *Oenothera* in preparation for the homoeotypic division. During prophase of this division the chromosomes become further condensed (figure 14), and at metaphase (figure 15), the daughter chromosomes are usually distributed in equal sets. Consequently a tetrad of microspores will generally have two nuclei with one chromosome number and two of another as determined by the heterotypic division, the sum of the two numbers being 21. Thus from the nucleus of figure 14 would have come two nuclei of a tetrad each with 9 chromosomes, and from the division of figure 15 two nuclei each with 12 chromosomes. Rarely are nuclei in tetrads found with 14 chromosomes (figure 16), but the union of gamete nuclei of this character produces the tetraploid.

Another factor leading to irregular distribution of chromosomes is the

lagging and consequent separation of occasional chromosomes from their proper groups. When this takes place on the heterotypic spindle there may be found during interkinesis 4 nuclei in a cell, two large and two small (figure 18), the latter containing the chromosomes that failed to reach the poles. Such small nuclei following the heterotypic division were not found to pass into the homoeotypic division. Similar small nuclei may be formed from chromosomes that lag during the homoeotypic division (figure 17). The final results of such failure of the normal distribution of chromosomes are sporocytes usually with 6 nuclei (figure 19), of which two are much smaller than the others. This gives the not uncommon condition of polyspores such as have been reported for other triploids and for various hybrid material.



TEXT FIGURE 3.—Samples of pollen from the tetraploid plant (27.36—18), parent of the tetraploid line. The 20 percent of good pollen showed grains much larger than those of *franciscana* (f), and with 4 or more lobes.  $\times 200$ , reduced one-third.

The behavior of the chromosomes during meiosis in the triploid makes clear the breeding peculiarities of this plant. As shown by the records of culture 27.36 (table 1), the progeny from the triploid plant was 3 tetraploids, 42 *franciscana*, 17 dwarfs with narrow leaves and small flowers, and 27 dwarfs that failed to flower. The large and strong plants were then products of the union of 14 chromosome gametes to give tetraploids and the union of 7 chromosome gametes to give *franciscana*. We do not know the chromosome organization in the dwarfs but they probably came by way of gametes with chromosome counts between 7 and 14. The 70 percent of shriveled pollen presented by the triploid would seem to be amply covered by the irregularities of chromosome distribution during meiosis, the smallest shriveled grains being from cells with nuclei of low chromosome

count. Nuclei with large chromosome numbers might also produce shriveled grains if the chromosome assortments were of an unfavorable character.

In connection with the large number of dwarfs there must also be taken into consideration the high mortality of plants in the field (culture 27.36, table 1) and the low seed germination of 22.6 percent. These data indicate that many gametic combinations fail to give viable zygotes or produce weaklings. The large number of *franciscana* plants thrown by the triploid shows that the segregation of the 7 chromosomes constituting the *franciscana* set must take place with much greater frequency than the segregation of two sets of *franciscana* chromosomes necessary for the gametes that may produce the tetraploid. This is to be expected since the accidents of irregular distribution are more likely to lessen the frequency of the diploid sets of 14 chromosomes than of the haploid sets.

#### CYTOLOGY OF THE TETRAPLOID

The cytology of the tetraploid has been studied from two plants (27.36-18 and 28.34-55) of the first and second generation. Stages from the first plant are given in figures 20-29 and from the second plant in figures 30-34. The account of meiosis will make use of both sets of figures for there was no difference in the behavior of the two plants. The equatorial plate of a somatic mitosis from a developing petal with its 28 chromosomes is shown in figure 20.

The chromosomes emerge from second contraction in short chains as illustrated in figure 21 where about 22 chromosomes are evident, the remaining 6 probably being hidden in the central chromatic mass. The chromosomes then become more evenly distributed in a stage corresponding to diakinesis, but they are still in short chains or occasional pairs as shown in figure 22 where 23 chromosomes are now quite free and only 5 remain in the central group. No examples were found of complete pairing during diakinesis which must take place rarely if at all. An early spindle of the heterotypic division is presented in figure 23 where the chromosomes appear some in pairs and some in short chains. There seemed to be no marked tendency to form tetravalents although the tetraploid must contain 4 sets of homologous chromosomes.

Metaphase of the heterotypic division is shown in figure 24 with most of the chromosomes regularly paired but with trivalents and unpaired chromosomes also present. Figure 30 from the second tetraploid is particularly interesting because all of the 28 chromosomes could be drawn from different levels; there appear to be 7 pairs, a chain of 5, a chain of 4, a chain of 3, and 2 chromosomes probably not associated. The appearance of groups of 4 and 5 chromosomes probably means that short chains have

not yet broken apart as pairs of chromosomes. The large number of chromosomes apparently makes it difficult for all to find synaptic mates and consequently odd chromosomes are frequently present. These conditions lead to irregularities of meiosis and must be responsible for much of the bad pollen found in the tetraploids.

No heterotypic metaphases were observed with all of the chromosomes paired but such pairing and subsequent disjunction or at least a regular segregation must take place since counts of 14 chromosomes were found in nuclei at interkinesis (figure 25). An early telophase of the heterotypic division is shown in figure 31 with 14 chromosomes in each daughter nucleus. Prophases of the homoeotypic division with 14 split chromosomes are illustrated in figures 26 and 27, and in figure 32 a metaphase is shown with 14 split chromosomes on each of the two spindles which lay at right angles to one another. A telophase of the homoeotypic division with 14 chromosomes is given in figure 33. These examples show conditions that would lead to the production of the very small amount of pollen with 14 chromosomes capable of reproducing the tetraploid line.

There is much distribution of chromosomes by the heterotypic division in counts above and below the number 14 but judging from the highly true breeding of the tetraploid such chromosome groups apparently rarely produce fertile pollen. The failure of chromosomes to find their synaptic mates is responsible as in the triploid for a large amount of lagging on the heterotypic spindles and this results in irregularities of segregation. Lagging also occurs in the homoeotypic divisions (figure 28), similar to that which has been described for the triploid, and small nuclei may be developed (figure 29), with the consequent formation of polyspores. There seems to be enough of these irregularities to account for the large proportions of shriveled pollen grains.

It is rather surprising that the tetraploid should present a smaller percentage of good pollen than the triploid. Perhaps the explanation lies in the larger number of chromosomes concerned which make it less easy for a chromosome to meet its appropriate mate. The second, third and fourth tetraploids of the line (table 1) apparently had better pollen than the first and also produced more seeds per capsule, but the fifth tetraploid was about as highly sterile as the first so there is no evidence of improved fertility in the line.

There is shown in figure 34 a sporocyte which apparently contains a restitution nucleus following the suppression of the heterotypic division, in which case the nucleus should contain 28 chromosomes. The chromatin is much distributed and there is no indication of the approach of the homoeotypic division. The example, however, suggests that irregularity characteristic of the haploid from *franciscana* (DAVIS and KULKARNI

1930) by which two pollen grains may be formed in a sporocyte following the suppression of the heterotypic division.

The stability of the tetraploid line is remarkable. The few variants that appeared (table 1) were almost all dwarfs that failed to flower. The parent *franciscana* so regularly thrown by the triploid has not been represented in the cultures of the tetraploid which means that it is difficult if not impossible to segregate a set of the 7 *franciscana* chromosomes from the 28 chromosomes or four sets in the tetraploid. The homologous chromosomes of the tetraploid endeavor to associate in pairs and there appears to be no opportunity for a single set of 7 to detach itself from so large a group. This will, however, happen in the triploid whenever a full set of trisomes are so placed on the heterotypic spindle that the third chromosomes of the groups all face one pole.

Variations of chromosome segregation in the tetraploid present themselves in numbers greater or less than 14 rather than between 7 and 14 as in the triploid. Such irregularities of chromosome distribution, expressed through these higher numbers, might be expected to result in greater disturbances of cell physiology with the consequent production of a higher proportion of shriveled pollen in the tetraploid than in the triploid, and this is markedly the case.

#### DISCUSSION

We have noted that the first triploids observed to arise directly from an *Oenothera* species were those from *Lamarckiana* established by chromosome counts of STOMPS (1912a) and LUTZ (1912). LUTZ also obtained triploids from *lata* and from *lata*  $\times$  *Lamarckiana*, and STOMPS (1912b) reported a triploid from the cross *biennis cruciata*  $\times$  *biennis*. These were important observations since they led to a series of studies which have established the fact that species of *Oenothera* not infrequently produce triploid sports in contrast to an early contrary opinion of GATES (1913). Later studies of which those on *Morus*, *Datura*, *Hyacinthus*, *Tulipa*, *Solanum*, *Zea*, *Crepis*, and *Petunia* are examples have shown that triploids are not rare in various groups of plants and support the conclusions on their importance as a stage in the development of higher polyploids.

The first study of the distribution at meiosis of the 21 chromosomes of a triploid *Oenothera* was that of GATES (1909b) on the hybrid *lata*  $\times$  *gigas*. He described a segregation of chromosomes during pollen formation usually of 10 and 11, but occasionally of 9 and 12. In a later paper on the cross *rubricalyx*  $\times$  *gigas* GATES (1923) again records the usual segregation as 10 and 11 with, however, irregularities that result from the lagging of chromosomes. In none of these studies on triploids did GATES describe or clearly figure the pairing of chromosomes at the time of the heterotypic division.

In this respect a paper of GEERTS (1911) on the cross *lata*  $\times$  *gigas* stands in contrast to the observations of GATES. GEERTS reported a pairing between two set of 7 chromosomes with 7 chromosomes unpaired both in vegetative cells and at the heterotypic division. The behavior, therefore, was described by GEERTS as of the *Drosera* type, but unfortunately the study was not developed in detail. VAN OVEREM (1922) for *semigigas* also reported as usual a grouping of chromosomes in 7 pairs with the remaining 7 in a scattered distribution. There is general agreement of these investigators and also of later workers with the conclusions of GATES that the more common segregation of chromosomes is that of 10 and 11, but genetical studies indicate that the small percentages of fertile gametes produced are chiefly in a group of lower chromosome numbers, 7 or 8, or in the diploid number of 14 chromosomes.

A triploid from *Oenothera pycnocarpa* stated by CATCHESIDE (1930) to segregate its chromosomes, usually 10 and 11 or more rarely 9 and 12, was also described as presenting at diakinesis a closed chain of 21 chromosomes. Such an arrangement, if true, would be fundamentally antagonistic to the theory of segmental interchange between non-homologous chromosomes as applied to *Oenothera* cytology and genetics. This account of a closed chain of 21 chromosomes was promptly challenged by DARLINGTON (1930b) and by CAPINPIN (1930), and DARLINGTON after an examination of CATCHESIDE's slides interpreted them as showing various associations of chromosomes in pairs, in trisomes, and in other arrangements including single elements. CATCHESIDE (1931), accepting the interpretations of DARLINGTON, has abandoned his position.

The first descriptions of trivalent chromosomes in *Oenothera* material were those of HAKANSSON (1926, 1930, 1931) for the triploid plant *Lamarckiana excelsa* and for certain trisomic derivatives from *Lamarckiana*. CAPINPIN (1930) is to report in detail on a number of triploids from *Lamarckiana* and other species such as *franciscana*, *ammophila*, *biennis* et cetera, none of the triploids being derived from crosses involving either triploid or tetraploid forms. CATCHESIDE (1931) in the revision of his studies on the triploid from *O. pycnocarpa* gives good figures of chromosome associations, many of them trivalents, and an excellent discussion of their significance as possible products from a structural hybrid. The present study describes trivalents as frequent in a triploid sport from the homozygous *franciscana*, a species with all pairing chromosomes.

Thus *Oenothera* in presenting trivalents at meiosis in triploids agrees with the studies on *Canna* (BELLING 1921), *Datura* (BELLING and BLAKESLEE 1922), *Hyacinthus* (BELLING 1925a, DARLINGTON 1929b), *Hemerocallis* (BELLING 1925b), *Campanula* (GAIRDNER 1926), *Solanum* (LESLEY 1926, JØRGENSEN 1928), *Zea* (RANDOLPH and McCLINTOCK 1926, Mc-

CLINTOCK 1929), *Prunus* (OKABE 1928, DARLINGTON 1928), *Tulipa* (NEWTON and DARLINGTON 1929), *Narcissus* (NAGAO 1929), *Crepis* (NAVASHIN 1929), *Lycoris* (INARIYAMA 1931), *Petunia* (DERMEN 1931, STEERE 1932). These accounts of meiosis in triploids report no conspicuous regularity in the association of the chromosomes to form trivalents. There are few figures that show complete sets of trivalents such as are presented by BELLING for *Canna* and *Hyacinthus*, by McCLINTOCK for *Zea*, by STEERE for *Petunia*, and illustrated for *Oenothera* in figure 5 of the present paper. The accounts generally describe and the figures present trivalents together with bivalents and univalents. Naturally the results of meiosis are most varied with the chromosomes segregated through a wide range of numbers. The lagging of chromosomes is common so that the full number frequently is not distributed to the two daughter nuclei following the heterotypic division and the lagging chromosomes may organize independent micronuclei. In consequence of these irregularities of chromosome distribution very large amounts of sterile pollen are formed.

With respect to the associations of chromosomes at meiosis in tetraploids they are bound to differ with the make up of the material whether auto- or allotetraploid. Allotetraploids with sets of dissimilar chromosomes are not likely to form quadrivalents at meiosis but autotetraploids with their four sets of homologous chromosomes may be expected to show tetrasomes. Tetravalents have been described and figured for *Datura* (BELLING and BLAKESLEE 1924), *Aucuba* (MEURMAN 1929), *Solanum* (JÖRGENSEN 1928, LESLEY and LESLEY 1930, LINDSTROM and KOOS 1931), *Prunus* (DARLINGTON 1928), *Hyacinthus* (DARLINGTON 1929b), *Pyrus* (DARLINGTON and MOFFETT 1930), *Dahlia* (LAWRENCE 1929, 1931), *Primula sinensis* (DARLINGTON 1931), *Rosa relict*a (ERLANSON 1931). The proportions of tetravalents present are widely various. *Datura* may present complete sets but in most material bivalents are found together with the quadrivalents which may be few in number.

The tetraploid from *Oenothera franciscana* failed to show more than occasional groupings of chromosomes in fours and these were in open chains at diakinesis (figures 22 and 23) and on the heterotypic spindle (figure 30). Such open chains readily break up into pairs which is the usual association of chromosomes in the metaphase of the heterotypic division. The failure of this autotetraploid to form marked tetrasomes and the predominance of pairs does not support the views on the importance of tetravalents, but rather strengthens the reports on other tetraploids where much irregularity of chromosome association is described. In this tetraploid *Oenothera* it is very common to find chromosomes which, having failed to meet with a mate, either associate with a pair to form a trisome (figure 24) or lie apart. The large numbers of chromosomes involved make probable a high per-



centage of such failures in synapsis with consequent irregularities of segregation.

Tetraploids from ring-forming species of *Oenothera*, which are structural hybrids, present conditions out of which disomes, tetrasomes, or chains might appear. Assuming *gigas* out of *Lamarckiana* (with its circle of 12 and one pair) to have the formula

ab	cd	ef	gh	ij	kl	mn
ab	cd	ef	gh	ij	kl	mn
bc	de	fg	hi	jk	la	mn
bc	de	fg	hi	jk	la	mn

the simplest arrangement of the chromosomes in synapsis would seem to be pairs such as ab ab, bc bc, cd cd, et cetera since both ends of each member of the pairs have homologues; the 4 chromosomes mn might be either as two pairs or as a tetrasome. However, chains might arise as for example cb ba ab bc which could be open or closed, short or long, possibly even up to the full number of the 24 chromosomes with interchanged segments. The only figures of *gigas* published are those of GATES (1911) and DAVIS (1911) and these indicate short chains and pairs during diakinesis and at metaphase of the heterotypic division. A re-examination of my slides supports these conclusions and the fact that *gigas* selfed gives a complex and diverse progeny indicates that the variants resulted from the numerous possibilities of irregular chromosome distribution.

In this account of the origin of a tetraploid from *Oenothera franciscana* by way of a triploid it is clear that the event must have taken place through the union of diploid gametes. There are only a few other accounts of the origin of tetraploid *Oenotheras* from hero plants, presumably triploids. DE VRIES (1923) gives the first record of a *gigas* individual by way of a plant of *semigigas* from a cross between *O. Lamarckiana* mut. *simplex* and *O. biennis* (Chicago). BOEDIJN (1924) reports *gigas* from *semigigas* plants out of the cross *lata* × *Lamarckiana*.

In contrast to the above are a number of accounts of tetraploid *Oenotheras* derived directly from diploid plants. The original *gigas* from *Lamarckiana* apparently had such an origin in the cultures of DE VRIES and other reports of *gigas* from *Lamarckiana* and from certain of its mutants give no suggestion of intermediary triploid plants before the appearance of the tetraploid. BARTLETT (1915a, b) obtained tetraploids directly from *O. stenomerus* and *O. pratincola*. DE VRIES (1918) recorded a tetraploid plant out of a line of *O. grandiflora*, also reporting *semigigas* from the same line, and *gigas* appeared in certain crosses; BOEDIJN (1924) added to this study. A tetraploid from *O. simplex nanella* of DE VRIES was established by BOEDIJN (1920). Other tetraploids have been reported by BOEDIJN as arising directly from crosses between hybrids and *gigas*. STOMPS (1925) ob-

tained a *biennis gigas* from a cross between *biennis* and *biennis cruciata*, and a *biennis cruciata gigas* from crosses of this tetraploid with the diploid *biennis cruciata*.

In view of the records of tetraploid *Oenotheras* out of diploid parentage it is not surprising that the hypothesis has been held by GATES and others of an origin after fertilization by a doubling of the chromosome number. This hypothesis of chromosome doubling is now best known through the extended discussion of WINGE (1917, 1932) who holds that a lack of harmony between the two sets of chromosomes in the  $F_1$  of a wide cross might be compensated for by an "indirect chromosome binding" of daughter chromosomes following a division somewhere in the germ path, possibly in the zygote. We can conceive such a doubling to be brought about (1) by the failure of a mitosis to separate its daughter chromosomes with the immediate organization of a restitution nucleus, (2) by the fusion of two daughter nuclei, or (3) by the union of two spindles of sister nuclei lying side by side to form one spindle (WINKLER 1916) as assumed by the "endo-duplication" hypothesis of JÖRGENSEN (1928). These possibilities have their chief interest in relation to the occasional appearance of stable tetraploids, which are in effect new species, in the  $F_1$  generations from crosses generally wide in character.

WINGE (1932) has assembled a number of cases of such stable tetraploids which apparently have arisen through the doubling of chromosome sets in the germ path of the hybrid. Good examples of this behavior are the cases of the tetraploid *Primula Kewensis* (NEWTON and PELLEW 1929), *Rosa Wilsni* (BLACKBURN and HARRISON 1924), *Nicotiana digluta* (CLAUSEN and GOODSPEED 1925), a tetraploid from the cross *Fragaria bracteata*  $\times$  *F. Helli* (ICHIJIMA 1926), *Saxifraga Potternensis* (MARSDEN-JONES and TURRILL 1930), *Brassica napocampestris* (FRANDSEN and WINGE 1932). Outstanding features of these "constant hybrids" are their fertility and the absence of segregation which result from the harmonious relations established between the sets of chromosomes now doubled.

Support for WINGE's hypothesis of the origin of constant hybrids through the doubling of chromosome sets in the germ path is offered in the remarkable experiments on the decapitation of stems in *Solanum*. Tetraploid shoots together with diploid arise from meristematic tissue in association with the callus over the wound (WINKLER 1916, JÖRGENSEN 1928, SANSOME 1930, LINDSTROM and KOOS 1931, and LINDSTROM 1932). LINDSTROM and KOOS present evidence of nuclear fusions in binucleate cells in the callus of decapitated haploids that may produce diploid shoots. An extensive literature reports the formation of nuclei with higher chromosome numbers in root tips and other tissues following experimental interference with mitosis through effects of chemicals, by wounding and by high

temperatures. Binucleate cells have been described and figured in a wide range of plant material. Tetraploid branches have appeared rather frequently in the extensive cultures of *Datura Stramonim* (BLAKESLEE and BELLING 1924). SCHWEMMLE (1928) has reported fertile tetraploid shoots on an  $F_1$  plant of *Oenothera Berteriana*  $\times$  *O. odorata*.

There are, however, serious objections to the acceptance of WINGE's hypothesis of "indirect chromosome binding" or somatic doubling as an explanation of the origin of those tetraploid *Oenotheras* that have arisen directly from diploids. By such origin the tetraploids should breed true and may be expected to be highly fertile since their nuclei carry two sets of homologous chromosomes brought together by the suppression of mitoses or through the fusion of sister nuclei either at rest or during simultaneous mitoses. Now such information as we have on the tetraploid *Oenotheras* out of diploids indicate that many of them were far from that condition of genetic stability to be expected on the hypothesis of the duplication of the chromosome sets. The *gigas* from *Lamarckiana* is well known to breed very far from true, and widely varied progenies were obtained from the Swedish *gigantea* of HERIBERT-NILSSON (1912) and the *gigas* from Palermo studied by GATES (1915). The *gigas* out of *stenomerus* (BARTLETT 1915a) produced 54 *gigas* and 9 variants in a culture of 102 plants; the remaining plants died or failed to flower indicating a high proportion of atypical forms. The *gigas* from *pratincola* (BARTLETT 1915b) gave 16 dwarfs in a culture of 25; the other 9 plants died. The *gigas* from *grandiflora* (DE VRIES 1918, BOEDIJN 1924) repeated the performance of the line in giving the forms *lorea* and *ochracea*. The *biennis gigas* (STOMPS 1925) threw dwarfs.

Thus we are forced to consider the probabilities of an origin of these tetraploid *Oenotheras* out of diploids through the union of diploid gametes which might result from the suppression of either the first or the second of the divisions of meiosis. The suppression of the first division permits of no segregation and the two diploid pollen grains formed give gametes which should carry the exact chromosome constitution of the diploid parent. Union of such gametes would give doubled sets of chromosomes and should a regular pairing result the tetraploid might breed true and the results would not be different from somatic doubling.

The suppression of the second division of meiosis also gives two diploid pollen grains but these will be genetically different when derived from heterozygous stock since the first division will have effected a segregation of chromosomes. There is less information on the suppression of the homoeotypic division than on the heterotypic which has been reported for material in many groups of plants and is not an uncommon phenomenon. Evidence of the suppression of the homoeotypic division is given for Pru-

nus (DARLINGTON 1930a), in hybrids of *Papaver* (YASUI 1931, figure 26), for *Triticum* hybrids (THOMPSON 1931), and for *Oenothera* (DAVIS and KULKARNI 1930, figures 36 and 37). HAKANSSON (1929) reports the fusion of homoeotypic spindles in *Salix viminalis*  $\times$  *S. caprea*. There is probably much more of this suppression of the second division of meiosis than this scant record would suggest; studies have not been particularly directed to it.

The species of *Oenothera* which have given tetraploids out of diploids are probably all chain-forming types in some degree and therefore structural hybrids. Tetraploids derived from them by way of diploid gametes formed through the suppression of the second division of meiosis could hardly fail to give indications through their breeding behavior of heterozygous conditions in the parent diploids. While the union of diploid gametes formed by the suppression of the first division of meiosis may be expected to give doubled sets of chromosomes we must grant the possibility of some variation among the gametes through rearrangements of chromosome segments in the prophases of meiosis and the consequent possibility of varied progeny. The evidence in the breeding records that have come to us indicates heterozygous conditions in the tetraploids under consideration. This is the argument for the conclusion that these tetraploid *Oenotheras* out of diploids have arisen through the union of diploid gametes and not by a process of somatic doubling.

#### SUMMARY

1. An autotetraploid of *Oenothera franciscana* Bartlett has arisen by way of a triploid in the  $F_2$  of the cross *franciscana* (*YTYT*)  $\times$  *franciscana sulfurea nana* (*yytl*).

2. The  $F_1$  of this cross was uniform, all plants (*YyTi*) were *franciscana*-like, yellow dominant over sulfur, tall over dwarf.

3. The  $F_2$  generation (table 1) gave the expected segregation of yellow tall, yellow dwarf, sulfur tall and sulfur dwarf through combinations of the gametes *YT*, *Yt*, *yT*, *yt*. Among the yellow tall were 4 "hero" plants (probably all triploids), one of which (26.51-477) was established to be a triploid, 21 chromosomes.

4. Since the tetraploid has failed to segregate either sulfur or dwarf this triploid must have arisen from the union of a 7 chromosome *franciscana* gamete (*YT*) with a 14 chromosome gamete (*YTYT*), the latter resulting from the suppression of the homoeotypic division of a *YT* nucleus and consequently including two sets of *franciscana* chromosomes.

5. The triploid (*YTYTYT*) selfed gave a progeny (table 1) of 42 *franciscana*, 44 various dwarfs, and 3 thick-leaved yellow tall plants (probably all tetraploids), one of which (27.36-18) was established to be tetrap-

loid, 28 chromosomes. The tetraploid must have come through the union of two 14 chromosome gametes and consequently carried four sets of *franciscana* chromosomes. The tetraploid has bred remarkably true through four generations (table 1).

6. The pollen of *franciscana* is 99 percent good; the capsules set about 500 seeds, 90 percent viable. The pollen of *franciscana sulfurea nana* is equally good, capsules set about 250 seeds, equally viable. The pollen of the F<sub>1</sub> plants was also 99 percent good, the capsules set about 500 seeds, and the germination was equally high.

7. The pollen of the triploid was about 30 percent good and about equally divided between 3- and 4-lobed grains (text figure 2); the triploid produced about 50 seeds to the capsule of which 22 percent proved to be viable.

8. The pollen of the tetraploid is about 20 percent well formed; the 4- or more lobed grains are larger than those of the triploid and very much larger than those of *franciscana* (text figure 3); the capsules set about 200 seeds moderately viable.

9. The 21 chromosomes of the triploid emerge from second contraction in pairs and in trisomes with occasional free chromosomes. If 7 trisomes are present they may lie so that 7 chromosomes pass to one pole and 14 to the other. Such segregation is not common but it must occur since nuclei were found in interkinesis and in the homoeotypic division with 7 and 14 chromosomes respectively (figures 8 and 16). Gametes with 7 chromosomes should on union give *franciscana* in agreement with the genetical findings. Union of 14 chromosome gametes should produce the tetraploid.

10. Most trisomes lie on the heterotypic spindle of the triploid with the third chromosome pointing sometimes to one pole and sometimes to the other giving various numerical distributions of the chromosomes (figure 5). There are also the pairs and odd chromosomes to effect irregularities of segregation together with lagging chromosomes that fail to reach the poles. Interkinesis in the triploid presents the split chromosomes characteristic of *Oenothera* but the lagging of chromosomes in the homoeotypic division frequently disturbs further the process of meiosis.

11. These irregularities of meiosis in the triploid would seem sufficient to account for the pollen sterility of about 70 percent and for the low seed germination of about 20 percent together with large numbers of dwarfs and the high mortality among the plants in the field since it is evident that many gametic combinations fail to give viable zygotes or produce weaklings.

12. The 28 chromosomes of the tetraploid are found mostly paired at metaphase of the heterotypic division but trisomes and odd chromosomes may be present and tetrasomes are occasionally represented by short

chains (figure 30). A regular segregation of the chromosomes does take place to a limited degree since counts of 14 chromosomes were found at various stages following the heterotypic division and from such nuclei must come the small percentage of fertile pollen.

• 13. There is much distribution of chromosomes through meiosis in the tetraploid in counts below the number 14 as a result of lagging and through failure of many chromosomes to find a synaptic mate. These irregularities are responsible for the large amount of bad pollen in the tetraploid which is greater than in the triploid, possibly because the larger number of chromosomes present makes it less easy for them appropriately to pair.

• 14. A sporocyte of the tetraploid was found (figure 34) which apparently contained a restitution nucleus following the suppression of the heterotypic division.

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## EXPLANATION OF PLATES

All figures were drawn with the aid of a camera lucida under the Zeiss apochromatic objective 1.5 (num. aper. 1.30) in combination with the compensating oculars K20 or K10. In reproduction the figures are reduced one-third from the magnification of 1600 diameters except for figures 18, 19 and 34 where the reduction is from 800 diameters.

## PLATE 1

FIGURE 1.—Triploid. Second contraction, synaptic associations indicated.

FIGURE 2.—Triploid. Chromosomes shortly after second contraction, a short chain of four is shown together with pairs.

FIGURE 3.—Triploid. The period corresponding to diakinesis. Trivalents are present together with bivalents.

FIGURE 4.—Triploid. Multipolar spindle of the heterotypic division showing trivalents and bivalents.

FIGURE 5.—Triploid. Metaphase of the heterotypic division with 7 trivalents. As arranged 12 chromosomes would pass to one pole and 9 chromosomes to the other.

FIGURE 6.—Triploid. Metaphase of the heterotypic division, 5 trivalents and 3 bivalents.

FIGURE 7.—Triploid. Anaphase of the heterotypic division, 11 chromosomes at one pole and 10 at the other.

FIGURE 8.—Triploid. Interkinesis, 7 split chromosomes.

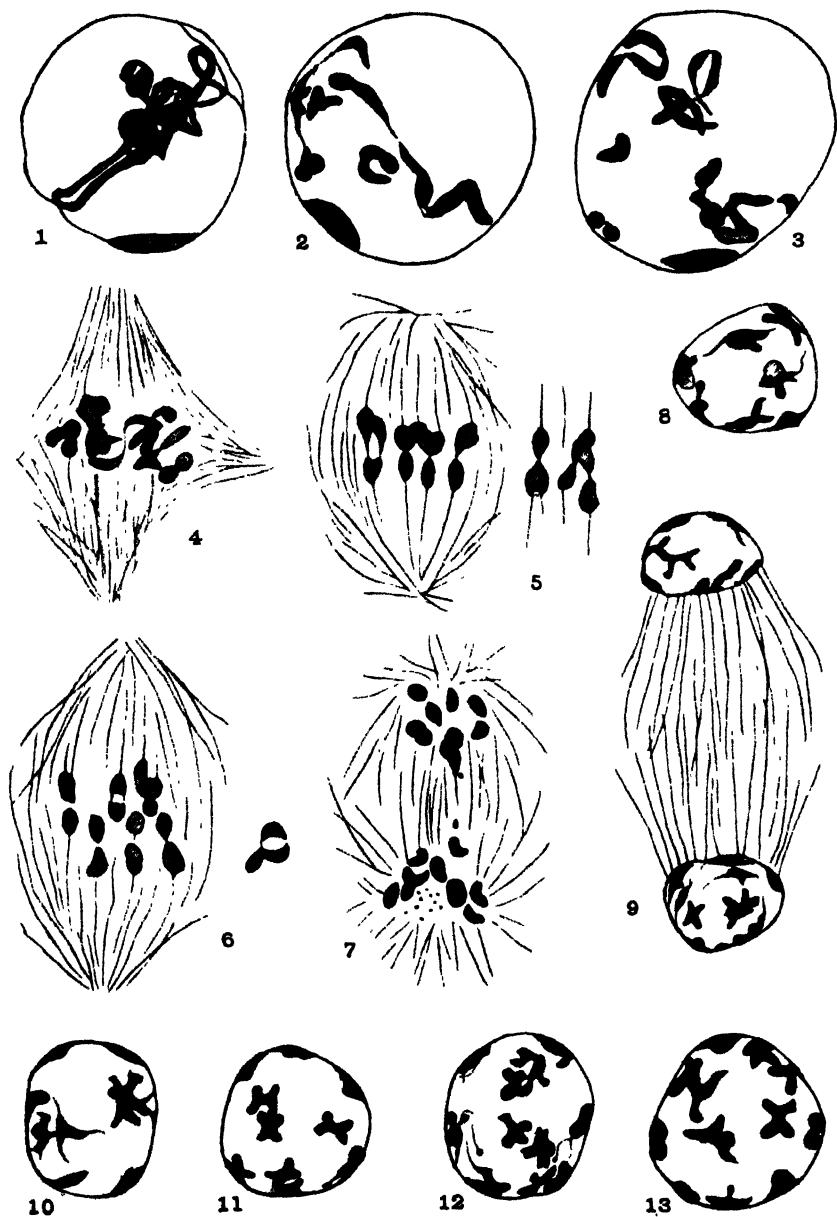
FIGURE 9.—Triploid. Interkinesis, 8 split chromosomes in one nucleus and 13 in the other.

FIGURE 10.—Triploid. Interkinesis, 9 split chromosomes.

FIGURE 11.—Triploid. Interkinesis, 10 split chromosomes.

FIGURE 12.—Triploid. Interkinesis, 11 split chromosomes.

FIGURE 13.—Triploid. Interkinesis, 12 split chromosomes.



## PLATE 2

FIGURE 14.—Triploid. Early spindle of homoeotypic division, 9 split chromosomes.

FIGURE 15.—Triploid. Metaphase of homoeotypic division, 12 split chromosomes.

FIGURE 16.—Triploid. Telophase of homoeotypic division, 14 chromosomes. The union of two gamete nuclei of this character would produce the tetraploid.

FIGURE 17.—Triploid. Heterotypic anaphase with lagging chromosomes which would probably have organized two micronuclei.

FIGURE 18.—Triploid. Interkinesis with two meganuclei and two micronuclei, the latter derived from lagging chromosomes such as are shown in figure 17.

FIGURE 19.—Triploid. Polyspores, the result of lagging chromosomes that form micronuclei.

FIGURE 20.—First tetraploid. Somatic mitosis from developing petal, 28 chromosomes.

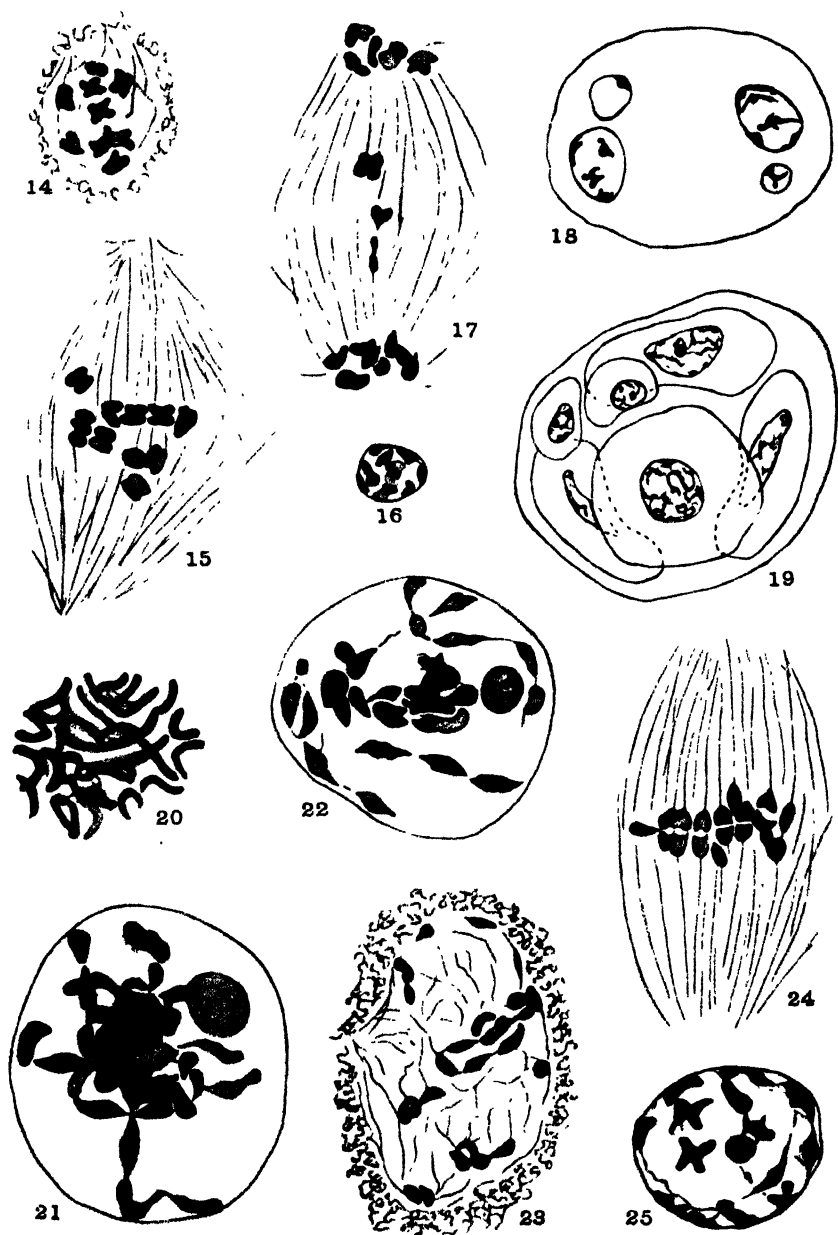
FIGURE 21.—First tetraploid. Chromosomes emerging from second contraction in short chains, about 22 evident.

FIGURE 22.—First tetraploid. Early diakinesis, chromosomes in short chains, 23 clearly defined, probably 5 in the central group.

FIGURE 23.—First tetraploid. Early spindle heterotypic division, chromosomes in pairs and in short chains.

FIGURE 24.—First tetraploid. Metaphase of heterotypic division, chromosomes mostly bivalents, some trivalents and univalents.

FIGURE 25.—First tetraploid. Interkinesis, 14 split chromosomes.



## PLATE 3

FIGURE 26.—First tetraploid. Prophase homoeotypic division, 14 split chromosomes.

FIGURE 27.—First tetraploid. Multipolar spindle homoeotypic division, 14 split chromosomes.

FIGURE 28.—First tetraploid. Homoeotypic telophase, lagging chromosomes between the daughter nuclei.

FIGURE 29.—First tetraploid. Mega- and micronuclei following the homoeotypic division, the former with 12 chromosomes, the latter probably formed from 2 lagging chromosomes.

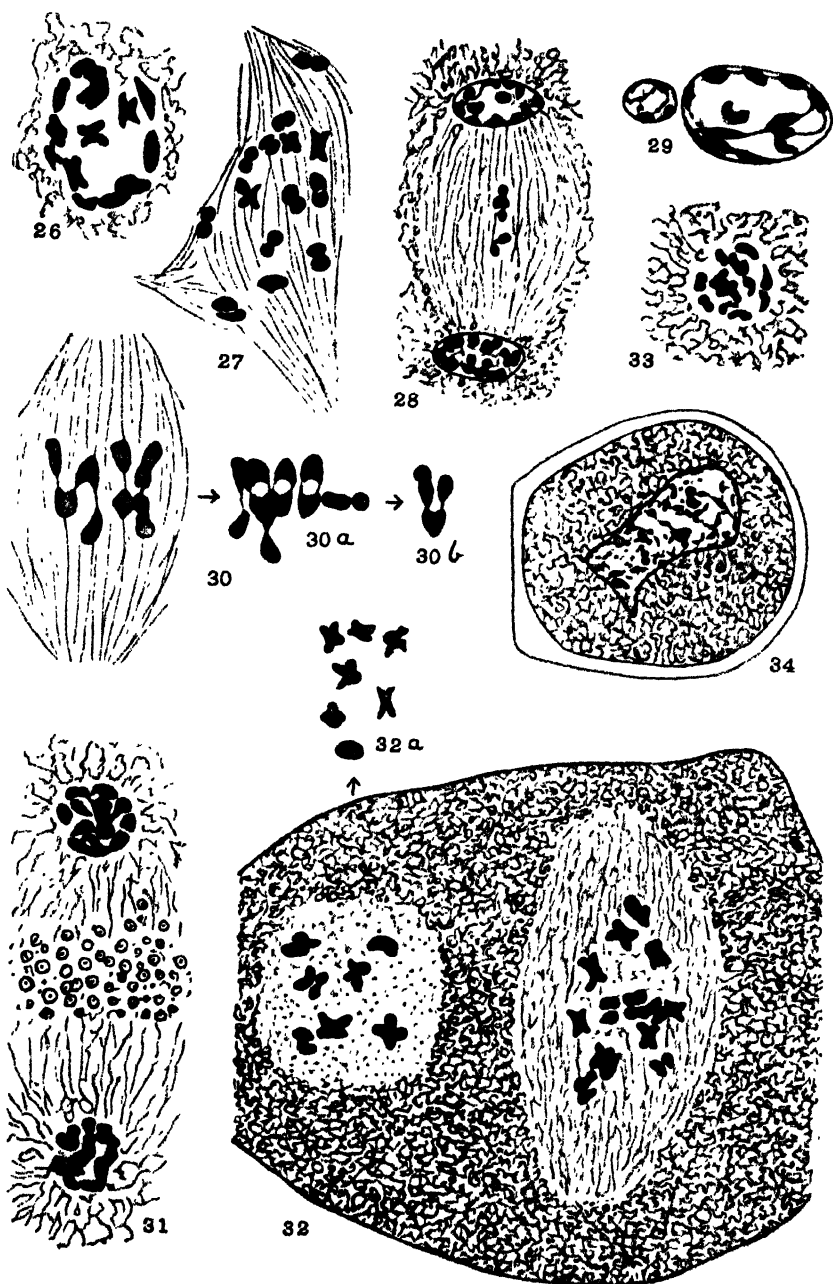
FIGURE 30.—Second tetraploid. Metaphase heterotypic division, the 28 chromosomes drawn at different foci; there appear to be 7 pairs, a chain of 5, a chain of 4, a chain of 3, and 2 chromosomes probably not associated.

FIGURE 31.—Second tetraploid. Early telophase heterotypic division, 14 chromosomes in each daughter nucleus, a zone of starch grains between the daughter nuclei.

FIGURE 32.—Second tetraploid. Early homoeotypic division the two spindles at right angles to one another, 14 split chromosomes on each spindle. Such a sporocyte should produce a tetrad of fertile pollen grains of the tetraploid genotype.

FIGURE 33.—Second tetraploid. Early homocotypic anaphase, 14 chromosomes. This should become the nucleus of a fertile pollen grain of the tetraploid genotype.

FIGURE 34.—Second tetraploid. Apparently a sporocyte in which the heterotypic division has been suppressed giving a very large restitution nucleus.



# GENETIC OBSERVATIONS ON THE GENUS LINARIA

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A few years ago, I obtained seeds from eighteen presumably different species of the genus *Linaria*—chiefly through the kindness of Professor Doctor E. BAUR and of HAAGE und SCHMIDT—in order to determine the value<sup>1</sup> of this group for genetical investigation. The list of species follows, together with some notes on their compatibility with each other.

1. *L. bipartita* Willd. Hab. northern Africa. Erect, branching, annual type. Fls. large, violet-purple, with orange palates above, becoming whitish toward the base. Spurs long and curved. Closely related to Nos. 7, 9, 10, 14, and probably will cross with them and give fertile hybrids. No crosses were obtained when the plants were used as female with Nos. 3 (12 pol.) and 17 (16 pol.).

2. *L. canadensis* Dumont. Hab. New Brunswick, New England, and south to southwest. Slender, erect, annual. Lvs. linear. Fls. small, violet-blue to purple. Late flowering. No crosses tried because of this point.

3. *L. Cymbalaria* Mill. (Kenilworth ivy). Hab. Europe. Four types grown, received under the names *vulgare* (trailing), *alba* (trailing with white flowers), *globosa* (bushy), and *compacta* (bushy). A trailing, glabrous plant, with reniform-orbicular, 5–9 lobed leaves. Fls. small, axillary, of various shades of purple above and of yellow at the lip. Spurs short. No crosses

<sup>1</sup> Earlier, I have made similar surveys of other genera; but, as no especially interesting contributions to genetic knowledge resulted, the results were not published, I now believe that this decision was a mistake. Short papers by geneticists who have had experience with various genera, setting forth the advantages and disadvantages of the material in question, might result in saving the time of other workers. I give two examples from my own experience.

A collection of 28 species of *Begonia* was made, and nearly 1000 interspecific crosses were attempted. Numerous cytological examinations were also made. Cytologically, the genus is poor material, the species having between 20 and 60 small chromosomes. In general, 1 hybrid was obtained for each 100 pollinations. No hybrids were obtained between the fibrous-rooted and the tuberous-rooted types. Hybrids between species belonging to different sections of the genus were not obtained. Even within the various sections hybridization was extremely difficult. Nevertheless, the plants have a distinct genetic advantage in ease of asexual reproduction; and, as is shown by the success of horticultural work, a considerable number of hybrids can be obtained between species that are closely related (presumably having the same chromosome number). A complete cytological survey of the genus would be very helpful to the horticulturist and possibly to the geneticist, though as genetic material the group is poor.

At another time, I made a large number of attempts at crossing on some 15 species of *Campanula*. Not a single hybrid was obtained. The genus can not be recommended to geneticists, therefore, though it is not too difficult cytologically, as the investigations of GAIRDNER (1926) and of DE VILMORIN and SIMONET (1927) show.

were obtained when the plants were used as female with Nos. 6 (34 pol.), 10 (29 pol.), 11 (61 pol.), 13 (24 pol.), 14 (3 pol.), and 18 (19 pol.). With No. 17, 6 capsules were obtained from 45 pollinations.

4. *L. dalmatica* Mill. Hab. Dalmatia, Oriens. Plants erect. Lvs. linear. Fls. yellow with deeper palate. Close to *L. macedonica*. Late-flowering. No flowers available for crossing with early-flowering species.

5. *L. genistifolia* Benth. Hab. southern Europe. Very similar to *L. vulgaris*, but no flowers of latter available for crossing.

6. *L. Hendersonii* (Hort.?). Similar to *L. vulgaris*, but with larger, showier flowers. Unable to find correct name or source of this type. Used as female with pollen of *L. vulgaris*, no capsules formed (8 pol.). Used as male, with *L. vulgaris*, 4 capsules were obtained out of 6 pollinations. In the latter case numerous seeds were formed containing embryos, which were probably defective, and very little endosperm. None of the seeds germinated.

7. *L. heterophylla* Desf. (*L. aparinoides* Dietr.). Hab. Mediterranean. The plants designated here were received under the name *L. aparinoides*; but they did not belong to this yellow-flowered species. Instead, they had purple flowers, with a little yellow on the palate. In all characteristics they stood close to *L. maroccana*. Used as female, no crosses were obtained with *L. Cymbalaria* (12 pol.) or *L. Broussonnetii* (*L. multipunctata*) (5 pol.).

8. *L. macedonica* Griseb. Hab. Macedonia. Broader leaves, otherwise like *L. dalmatica*. Two varieties were grown, differing only in shade of flower color. They were received under the varietal names *speciosa* and *nympha*. Used as female, no crosses were obtained with *L. Broussonnetii* (7 pol.) or *L. reticulata* (8 pol.).

9. *L. macroura* Link. Hab. southern Russia. LINK gives no description in Enum. Hort. Berol. II, p. 137. He refers to MARSHALL (1819) Flora taurico-causica III: p. 413; but this description only designates the plant as erect and the leaves as linear and alternate. I have no idea whether the plants received under this name are true *L. macroura* or not. They have purple flowers and are very close to *L. maroccana*, though they are late-flowering. No flowers were available for crossing when the majority of the species were in flower.

10. *L. maroccana* Hook. Hab. Marocc. Erect, annual. Lvs. linear, sometimes whorled, slightly hairy. Fls. violet to reddish-violet. Used as female, no crosses were obtained with Nos. 3 (43 pol.) or 11 (13 pol.).

11. *L. Broussonnetii* Chav. (*L. multipunctata* Hoffm. & Link). Hab. Marocc.; Lusit. This species and the one received under the name *L. Perezii* are closely related. The yellow flowers are spotted with copper red



flecks. Used as female, no crosses were obtained with Nos. 3 (19 pol.), 10 (11 pol.), 14 (8 pol.), and 17 (9 pol.). Hybrids were obtained with pollen of *N. Perezii*.

12. *L. Pancicii* (Hort.). Apparently not *L. Pancicii* of Janka. Erect, annual. Lvs. linear. Fls. medium size, light yellow. Flowered late. No opportunity to test compatibilities with other species.

13. *L. Perezii* Gay. (Apparently is *N. Tournefortii* Steud.) Hab. southwestern Europe. Short, erect plant, apparently short-lived perennial. Lvs. narrow lanceolate, short. Fls. pale yellow. Used as female, no crosses were obtained with Nos. 3 (64 pol.), 7 (5 pol.), 8 (4 pol.), 10 (8 pol.), 14 (12 pol.), 15 (3 pol.), 17 (14 pol.), and 18 (16 pol.). Hybrids obtained with *L. Broussonnetii* reciprocally.

14. *L. reticulata* Desf. Hab. northern Africa. Erect annual. Lvs. linear, whorled. Fls. yellow with palate copper-colored; above netted with purple veins. Used as female, no crosses obtained with Nos. 3 (25 pol.), 11 (10 pol.), and 17 (19 pol.). Hybrids with *L. sapphirina* reciprocally, and with *L. maroccana* used as male.

15. *L. sapphirina* Hoffm. & Link (*L. delphinoides* Gay). Hab. northern Africa. Erect annual similar to *L. maroccana*. Flowers a somewhat lighter violet-rose. Used as female, no crosses were obtained with Nos. 13 (25 pol.) or 17 (11 pol.). Hybrids obtained when *L. reticulata* was used as male.

16. *L. triornithophora* Willd. Hab. Lusitan. Erect perennial. Lvs. lanceolate in groups of 3 or 4. Fls. in groups of 3; purple-striped with orange palate. Spurs inflated. Flowers late. No opportunity to test compatibilities with other species.

17. *L. triphylla* Mill. Hab. Reg. Mediterranean. Lvs. oval in threes. Flowers bright yellow. Used as female, no crosses were obtained with Nos. 3 (27 pol.), 6 (14 pol.), 10 (9 pol.), 11 (13 pol.), 13 (17 pol.), 14 (9 pol.), 18 (11 pol.).

18. *L. vulgaris* Mill. The common butter and eggs. Adv. from Europe. Erect perennial. Lvs. long, linear. Fls. yellow, darker on bearded palate.

Chromosome counts were made on several species (see HERTZ 1927). *L. Cymbalaria* has 14 chromosomes (somatic). These numbers were checked in root-tips and in buds. No lagging chromosomes. *L. heterophylla* (*L. aparanioides*) shows 6 pairs of oval chromosomes of approximately the same size at IM. No lagging. *L. macroura* shows 6 pairs of chromosomes, 4 pairs being oval and 2 pairs slightly elongated. No lagging. *L. maroccana* shows 6 pairs of oval chromosomes having approximately the same size. *L.*

*Broussonnetii* (*L. multipunctata*) shows 6 pairs of oval chromosomes having approximately the same size. One pair occasionally lags at IA. *L. Pancicii* shows 6 pairs of oval chromosomes of approximately the same size. One or two pairs frequently lag at IA. *L. Perezii* shows 6 pairs of chromosomes of approximately the same size. Several plates showed that one pair sometimes precedes the others to the poles. *L. reticulata* shows 6 pairs of chromosomes, of which 2 pairs are one and one-half times as long as the others. *L. triphylla* shows 6 pairs of chromosomes commonly, though several plates were counted with 7 pairs. *L. vulgaris* shows 6 pairs of chromosomes, of which one pair is somewhat larger than the others.

These results indicate that the *Linaria* species have six pairs of chromosomes with the exception of *L. Cymbalaria* (seven pairs). It is thus an example of a genus where marked sexual incompatibility has developed apart from changes in chromosome number. *L. Cymbalaria* has been given generic status, largely on account of its reniform-orbicular leaves. But there appears to be no valid genetic reason for such a separation. Though *L. Cymbalaria* does not hybridize with other species, the same statement may be made about several of the six-chromosome forms. Moreover, the pollen of several six-chromosome species grows normally in styles of *L. Cymbalaria*; and when the pollen of *L. triphylla* was applied, artificial parthenogenesis resulted, the plants being maternal diploids.

The four varieties of *L. Cymbalaria* cross together readily, and the resulting hybrids are fertile. The trailing character is dominant to the *globosa* or *compacta* character. The purple flowers of the common type are dominant to the white flowers of the *alba* variety.

*L. sapphirina* (*L. delphinoides*), *L. maroccana*, and *L. reticulata*, if the types I have are correctly designated, are simply varieties of the same species, crossing together freely in reciprocal crosses, and producing hybrids which exhibit no diminished fertility. *L. bipartita* and *L. macroura* apparently belong to the same group. This is perhaps not strange, as all of these species are found in various places around the border of the Mediterranean Sea.

The above species have genetic differences affecting flower color as follows:

*P* is a gene producing purple color in presence of *C*, a carminea gene hypostatic to *P*. *I* is an intensity gene. *PCI* gives a bright purple flower; *PCi* gives a pale purple flower. *V* is a gene that does not affect the intensity of color when present with *PCI* or *PCi*; but *v* with *PCI* tends to reduce the intensity of the purple color, and with *PCi* gives very light lavender-colored flowers and extends the yellow color of the palate. An additional gene, *A* (or its allelomorph) reduces the color in the spur and makes the color of the

palate a very light yellow. I was unable to determine the relationship of this pair.

Another group of species, which may be called yellow-flowered species, exhibited some sexual compatibility. The crosses *L. Hendersonii* × *L. Perezii* and *L. vulgaris* × *L. Hendersonii* set seeds which contained embryos; but the seeds did not germinate. Matings between *L. Perezii* and *L. Broussonnetii* (*L. multipunctata*) produced hybrids reciprocally. The hybrids were indistinguishable, irrespective of the way the cross was made. In habit of growth, color of flowers (with the exception of the copper-colored flecking, which was dominant), and shape and size of the leaves, the hybrids were strictly intermediate between the parental types. The hybrids were completely sterile.

One is forced to conclude that the genus *Linaria* will not be very useful in solving genetic problems.

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# THE INHERITANCE OF VIRESCENT YELLOW AND RED PLANT COLORS IN COTTON<sup>1</sup>

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## PREVIOUS WORK ON CHLOROPHYLL DEFICIENCIES

Deficiencies in the amount of chlorophyll in the cotton plant have been reported in relatively few instances. BALLS (1908) made a cross between an upland cotton with light green leaves and a dark green Egyptian cotton. He states that in the F<sub>2</sub> generation, plants with light green leaves and plants with dark green leaves appeared in a 3:1 ratio. STROMAN and MAHONEY (1925) recovered two chlorophyll deficient types in the F<sub>2</sub> generation from crosses between upland and Egyptian cottons (*Gossypium hirsutum* × *G. barbadense*). The first type, yellow seedlings, was due to 2 recessive genes, the segregating plants producing 15 green:1 yellow seedling. The second type was a pattern seedling character in which certain irregular areas devoid of chlorophyll on the cotyledon leaves were surrounded by normal green pigment. These pattern characters were inherited as recessives. In some cases two, in others three, pairs of genes were concerned. HARLAND (1932) also recovered from several different interspecific crosses various types which were partially deficient in chlorophyll. HORLACHER and KILLOUGH (1931) induced in upland cotton (*G. hirsutum*) by radiations a type that produces yellow seedlings lacking in chlorophyll. This yellow is lethal and a simple recessive to green, heterozygous plants producing 3 green: 1 yellow seedling. The yellow seedlings die after using up the stored food material of the seed. In all such chlorophyll deficient types which have been observed by the authors, the deficient portions are yellow due to the presence of carotinoid<sup>2</sup> pigments and the absence of green chlorophyll. No portion of a cotton plant devoid of carotinoid pigments was observed. The yellow seedling character mentioned above is not to be confused with the virescent yellow mature plant color discussed in this paper.

<sup>1</sup> Contribution from the TEXAS AGRICULTURAL EXPERIMENT STATION, College Station, Texas, Technical Paper No. 237.

<sup>2</sup> The authors wish to express their appreciation to Dr. R. G. REEVES, Professor of Biology, for conducting chemical and microchemical tests which show that the yellow pigment in the cotton plant is due to the two carotinoid pigments, carotin and xanthophyll.

## HISTORY AND DESCRIPTION OF VIRESCENT YELLOW

The authors are indebted to Mr. R. E. KAPER for the stock of virescent yellow cotton, which was found growing on the TEXAS AGRICULTURAL EXPERIMENT STATION, Substation No. 7, Spur, Texas, in 1925. Only two plants of this kind appeared among thousands of normal green cotton plants growing in a large field of Mebane cotton. From the nature of its discovery, it would appear probable that its origin was the result of a recent mutation. The senior author has grown numerous progenies from the original material which have always bred true for the virescent yellow plant color.

The seedlings and young plants of virescent yellow cotton have a greenish yellow appearance due to a partial deficiency of chlorophyll. The yellow carotinoid pigments are not completely masked by the green chlorophyll that is present, thus giving the plants their characteristic appearance. As these plants grow older the chlorophyll increases in amount to such an extent that at maturity virescent yellow plants are less striking in appearance and not as readily distinguishable from the normal green plants. Frost gives to the virescent yellow plants a yellowish cast in the autumn.

VIRESCENT YELLOW  $\times$  GREEN

In crosses we have made between virescent yellow and normal green cotton, the plants of the  $F_1$  generation are all green. The  $F_2$  generation segregated into 602 green:196 virescent yellow, a deviation of only  $3.5 \pm 8.26$  from a 3:1 ratio. The genes concerned have been designated  $V$ , green, and  $v$ , virescent yellow.

## RED LEAF COTTON

The occurrence and heredity of anthocyanin pigments in the cotton plant have been studied by several investigators. There are two general types of distribution of this pigment.

The first type is the red plant color which is produced by the anthocyanin and distributed throughout the stem and leaves making the entire plant red. This type has been described in *G. hirsutum* by McLENDON (1912), THADANI (1921), WARE (1927, 1929), and CARVER (1929). It has also been described by LEAKE (1911) in *G. arboreum*. It is usually called red leaf cotton.

The second type is the red leaf spot which is confined to the leaf pulvinus. This spot is due to the development of anthocyanin in the epidermal and sub-epidermal cells of the petiole at the point where it divides into the leaf-veins. This type has been described by BALLS (1908, 1910) in *G. barbadense*. This red spot on the leaf is characteristic of all the so-called green

varieties of *G. hirsutum*, and of many other species of *Gossypium*. In this paper this type is classed as green leaf cotton.

The red plant color produced by the complete distribution of anthocyanin throughout the plant has been found to be a simple dominant to green plant color by each of the investigators mentioned. BALLS reports that the red leaf spot is also a simple dominant to green.

The anthocyanin pigment in red leaf cotton is distributed over the entire plant, but develops in greater quantities in those parts which are directly exposed to the rays of the sun. Less anthocyanin develops on the under sides of the branches, petioles, and leaves than on the upper sides. When these surfaces are turned over and held in position so that the sun's rays strike these under surfaces directly, they also develop as much red color as is normally characteristic of the upper surfaces. The effect of light on the development of anthocyanin pigment is further shown by the fact that plants of the red leaf strain grown in the greenhouse during the winter develop very small amounts of red pigment, giving to the plants only a slightly reddish cast on a green background, whereas plants of the same genotype grown in the field in the summer are solid red in appearance, except for the greenish underparts noted above.

When red leaf cotton is placed in an alkaline solution the anthocyanin pigment turns blue. The red color is restored when the tissue is placed in an acid solution.

#### RED LEAF $\times$ GREEN

Our crosses between red leaf cotton and green leaf cotton, which has the red leaf spot, have confirmed the interpretation of the inheritance of these two characters given by the investigators mentioned above. The  $F_1$  generation from this cross was red, but of a lighter shade than the red leaf parent. The  $F_2$  generation consisted of 357 red:122 green, a deviation of only  $2.25 \pm 6.39$  from a 3:1 ratio.

The homozygous red ( $RR$ ) plants can usually be separated phenotypically from the heterozygous red ( $Rr$ ) plants. In general the  $RR$  plants are dark red and the  $Rr$  plants are light red. The genotype of any red leaf plant cannot, however, be determined definitely without a progeny test.

#### VIRESCENT YELLOW $\times$ RED LEAF

Virescent yellow cotton was crossed with red leaf cotton. The plants of the  $F_1$  generation were light red. In the  $F_2$  generation red leaf and virescent yellow plants were produced, as also were green plants and a new type which has been named bronze. These types appeared in the proportions shown in table 1.

TABLE 1  
*F<sub>2</sub> population from Virescent Yellow × Red Leaf Cotton.*

	RED LEAF	BRONZE	GREEN	VIRESCENT YELLOW
Observed	256	101	94	28
Calculated, 9:3:3:1	269.44	89.81	89.81	29.94
o-c	-13.44	11.19	4.19	-1.94

$p = .50$

It is apparent from table 1 that two pairs of genes which are inherited independently are concerned in this cross. The genotype of the original red leaf parent was *RRVV*, that of the virescent yellow *rrvv*. The green segregates were *rrVV* or *rrVv*. The bronze segregates were due to the action of the red leaf gene *R* on virescent yellow and were of the genotypes *RRvv*, which is dark bronze, and *Rrvv* which is light bronze. All four pigments, anthocyanin, chlorophyll, xanthophyll, and carotin, are present in bronze plants and combine in such proportions as to produce this color type. Mature bronze plants are practically indistinguishable from red leaf plants. In general, the *F<sub>2</sub>* red leaf plants can be divided phenotypically into dark red and light red, and the *F<sub>2</sub>* bronze plants can be divided phenotypically into dark bronze and light bronze. However, in neither case can the separation of the genotype be made with absolute certainty without resort to the breeding test. *F<sub>3</sub>* progeny were grown from self-fertilized bolls of 40 different *F<sub>2</sub>* plants with the results given in table 2.

TABLE 2  
*Breeding behavior of selfed F<sub>2</sub> segregates.*

<i>F<sub>1</sub></i> PHENOTYPE	<i>F<sub>2</sub></i> GENOTYPE	NO. OF <i>F<sub>2</sub></i> PLANTS	PHENOTYPIC CLASSES IN <i>F<sub>3</sub></i>					
			DARK RED	LIGHT RED	DARK BRONZE	LIGHT BRONZE	GREEN	VIRESCENT YELLOW
dark red	<i>RRVV</i>	3	66					
dark red	<i>RRVv</i>	2	30		5			
light red	<i>RrVV</i>	1	5	21			12	1 (?)
light red	<i>RrVv</i>	11	38	92	17	34	45	12
dark bronze	<i>RRvv</i>	2			50			
light bronze	<i>Rrvv</i>	8			33	85		43
green	<i>rrVV</i>	0						
green	<i>rrVv</i>	10					174	64
virescent yellow	<i>rrvv</i>	3						35

In each case the breeding behavior of the  $F_2$  genotype is according to expectation, with the exception of the one virescent yellow plant appearing among the progeny of the light red  $F_2$  plant, which, according to the remainder of its progeny, must have been  $RrVV$ . It seems probable that this one off-type plant in the entire  $F_2$  population of 862 was the result of mechanical mixture.

The  $F_2$  population from selfing the light red plants heterozygous for both genes,  $RrVv$ , fits very closely the expected ratio of 3 dark red : 6 light red : 1 dark bronze : 2 light bronze : 3 green : 1 virescent yellow (table 3).

TABLE 3  
*F<sub>2</sub> population from RrVv Light Red F<sub>2</sub>'s.*

	DARK RED	LIGHT RED	DARK BRONZE	LIGHT BRONZE	GREEN	VIRESCENT YELLOW
Observed	38	92	17	34	45	12
Calculated, 3:6:1:2:3:1	44.62	89.25	14.87	29.75	44.62	14.87
o-c	-6.62	2.75	2.13	4.25	0.38	-2.87

$p = .77$

The different color types of cotton plants resulting from this dihybrid cross can more readily be distinguished when the plants are bearing their first few adult leaves than at any other time in the life of the plants. It is impossible to classify such plants with any degree of accuracy at later stages of growth. Neither is classification from the cotyledon leaves accurate.

#### SUMMARY

1. Virescent yellow cotton, a new type, is described. This cotton is greenish yellow when young. The chlorophyll gradually increases in amount so that at maturity these plants are not readily distinguishable from normal green plants. Virescent yellow is a simple recessive to green. The genes of this pair have been designated as  $V$  (green) and  $v$  (virescent yellow).

2. Red leaf cotton is produced by the distribution of anthocyanin pigment throughout the plant. Data are presented, confirming the results secured by others, which indicate that red leaf  $R$  is a simple dominant to green leaf  $r$ .

3. Genes  $R$  and  $V$  are inherited independently.

4. The combination of  $R$  with  $v$  produces a new type named bronze. Bronze is produced by the development of red anthocyanin pigment on a virescent yellow background.



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# FLEXED TAIL IN THE MOUSE, *MUS MUSCULUS*

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In January 1927 the writer found two albino males with markedly flexed tails. These tails were rigid at the bends, so it was evident that vertebral fusions, or at least some anatomical peculiarity other than muscular contraction, was the cause. The mutation was found in a stock which had descended from animals supplied by Dr. W. E. CASTLE of HARVARD UNIVERSITY. The abnormal males were mated immediately with normal albino females, and a flexed tailed stock was started from the flexed animals of the  $F_2$  generation. The same mutation, or at least something resembling it in appearance, has been found in the colony several times since the original discovery.

Subsequent experience has shown that the character flexed tail is highly variable (figures 1 and 2). As a rule there are one or more permanent angles in the tail, though sometimes as many as five. They may be acute, obtuse, or right angles, and are most frequent in the proximal half of the tail, though at times one occurs near the tip. Rarely the tail turns sharply cephalad over the rump, then bends abruptly backward. This was the case in one of the two original mutant males, though, curiously enough, his numerous descendants rarely show this particular form of flexure. Curves of varying extent are sometimes found instead of the sharp angular bends, and spirals are fairly frequent. The latter are usually, but not always, near the base of the tail. They range from very tight close twists to widely open forms, and their direction is either clockwise or counterclockwise. In addition to being flexed, the tail is sometimes conspicuously shortened. The tail is usually very stiff where angles, curves, or spirals occur, and attempts to straighten it are likely to result in a break at that point. Sometimes there is no visible flexure, but palpation reveals rigid areas of varying extent. These stiff segments in straight tails may be so limited in length, and approach the normal so closely in flexibility, that considerable experience is required to decide whether or not the animal should be classified as flexed. Thus flexed tails range from extremely contorted or shortened forms to normality, and individuals show a variety of combinations of the characteristics mentioned.

Flexed tailed animals would certainly be handicapped under natural

<sup>1</sup> Mr. MIXTER secured the data on inbreeding. Miss PERMAR's contribution is noted in the text. Mr. HUNT planned and executed all the remaining experiments, and prepared the manuscript.

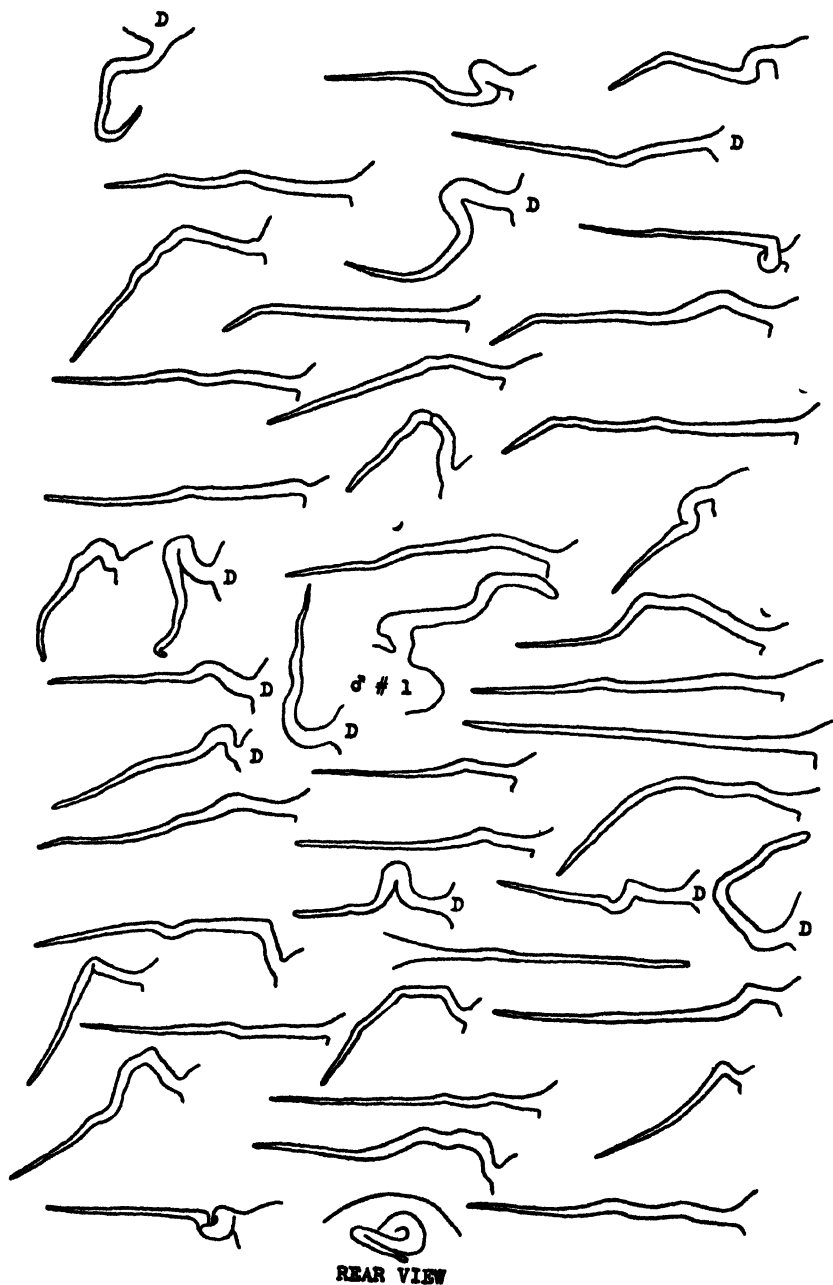


FIGURE 1.—Variations in the flexed character among closely related individuals. Male 1 (shown at the center) was mated with two normal females (crosses 1a and 1b), then with four of his resulting  $F_1$  daughters (crosses 26a, 26b, 26c, and 26e). The flexed tails from the progeny of the latter crosses are shown in the figure. D at the base of a sketch means that it is a dorsal view, one figure gives a rear view, and all others show the right or left side.

conditions. The shredded paper bedding must be torn into short lengths, otherwise the hooked appendage of some of the mice gets hopelessly tan-

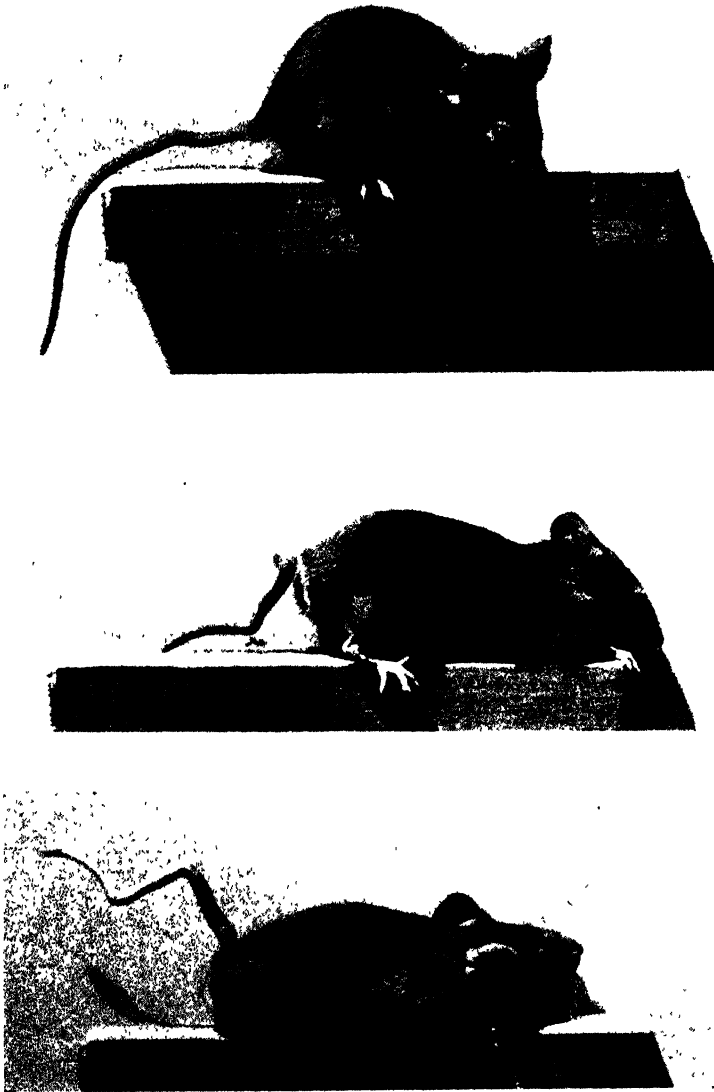


FIGURE 2.—Flexed tailed mice. The middle photograph shows marked shortening of the tail and a tendency to form a spiral at its base.

gled in it, with the result that death may come before the accident is discovered. It is evident that such extremely abnormal mice would be handicapped greatly in the wild when running from their enemies.

Several other abnormalities appeared among the flexed tailed animals. They were obviously anemic at birth. The blood was studied and the results of this investigation will appear in a later paper. The right, left, or both eyes were closed in some cases. This frequently occurred among several young from the same mating, though the exact nature of the defect and its mode of inheritance were not investigated. Some flexed tailed mice showed a dorsal enlargement of the head in front of the ears, suggesting hydrocephaly.<sup>2</sup> The desirability of concentrating our efforts upon the study of the complex phenomena of flexed excluded the analysis of these other traits. The eye defect particularly merits further study.

#### REVIEW OF LITERATURE

Several investigators have observed flexions in the mouse's tail resembling, or identical with, our mutation. PLATE (1910) studied such a character, and found it to be hereditary. BLANK (1916, 1917) investigated the embryological development and morphology of bent tail in the mouse. His material consisted of 45 mutant mice from PLATE's breed, together with 6 embryos from the uterus of a female. He found that a flexure was due to a lateral fusion between two adjacent vertebrae, which thrust the vertebral epiphyses with their growth zones, the intervertebral disc, and the vestige of the notochord toward the opposite side of the tail. The presence of a flexure demonstrated the existence of a fusion, but there might be a vertebral union without a flexure. BLANK believed that the inheritance of bent tail did not follow Mendel's Law, but his published quantitative data, as far as they go, support our contention that flexed tail is a simple Mendelian recessive character.

DOBROVOLSKAIA-ZAVADSKAIA has reported variations in the tail of the mouse which were encountered in experiments designed to produce changes in the germ-plasm by means of X-rays. Two types were obtained: a waltzing mouse whose trait proved to be recessive, and a dominant short tailed type which continually mutated, producing tailless, filiform tailed, kinky or bent tailed, helicoid, et cetera, variants.

DANFORTH (1930) observed kinky tails in a strain of mice having hereditary duplication of posterior parts (extra pair of hind legs, two rectums, two urethrae, two bladders, four kidneys, four gonads, four pubic bones, doubled intestine, bifurcated spinal cord, et cetera). A genetic explanation for this kinky tail was not offered.

#### MISS PERMAR'S EXPERIMENTS

Miss DOROTHY PERMAR (1928) began a study of the mode of inheritance of flexed tail during the fall of 1927 in the zoological laboratory at MICH-

<sup>2</sup> Since the completion of this manuscript, F. H. CLARK at HARVARD has reported on the inheritance of this hydrocephaly of the flexed tailed mouse (Proceedings of the National Academy of Sciences, vol. 18, pp. 654-656, Nov., 1932).

GAN STATE COLLEGE. Crosses were made between flexed animals to determine whether the trait breeds true. Flexed and normal mice also were mated, and the resulting  $F_1$  generation bred  $F_2$ 's.

Fourteen pairs of flexed animals produced 127 young, all of which were flexed tailed. In two additional matings one parent was stiff tailed and the other flexed; all 10 young from these two crosses were flexed. Thus the flexed tailed character bred true, like a simple Mendelian recessive, and stiff tailed animals (those in which the tail was rigid in one or more places but did not show an angular bend) bred like the flexed animals.

Thirteen crosses were made between flexed tailed and normals. In five of these crosses the male was the flexed parent and the female was normal. Eight reciprocal crosses also were bred. All the 142  $F_1$  generation young had normal tails. Thus it appeared that the flexed character was recessive to normality.

Thirty matings between  $F_1$  parents produced an  $F_2$  population of 1,065. In most of these crosses both parents had been bred by the same pair of  $P_1$ 's, and frequently both the  $F_1$ 's of a pair came from the same litter. Nine hundred thirty-six of the  $F_2$ 's were normal tailed and 129 were flexed, a ratio of 7.26 normals to 1.00 flexed. The flexed  $\times$  flexed and the flexed  $\times$  normal matings gave results which were in perfect agreement with the hypothesis that flexed tail is a simple Mendelian recessive character, but the  $F_2$  distribution was decidedly inconsistent with this view. No other satisfactory genetic explanation was found, so that Miss PERMAR's work failed to reveal the mode of inheritance of the flexed tailed mutation.

It was obvious that a fresh start was necessary. Certain facts discovered during Miss PERMAR's work suggested why her  $F_2$  ratio deviated so markedly from 3:1. A flexed tail is rigid at and near the bends and sometimes the flexure is absent but the rigidity remains. Thus unless the tail were palpated it might pass for normal. Furthermore, stiffened but flexureless tails occasionally have such exceedingly short rigid segments that a hasty examination might lead one to classify them as normal. The writer has found cases in which short rigid segments can be made pliable by gently bending them two or three times between the thumb and finger. Thus the flexed character grades into normality so perfectly that great care must *always* be used in differentiating between a non-flexed but stiff tail, and one which is entirely normal. Indeed, as will be shown later, homozygous flexed animals occasionally have a tail which can not be distinguished from a normal one, so that the genetic character of the mouse can be demonstrated only by its ancestry and by breeding tests. It is therefore probable that Miss PERMAR's deficiency of flexed tailed animals in the  $F_2$  generation was in part due to classifying a few animals as normal which were actually flexed.

A second complicating factor was the anemia of the newborn flexed. It was discovered late in Miss PERMAR's work that there were anemic and normal blooded mice in the  $F_2$  litters. Mice are hairless at birth, the only color they show being the red which is due to the blood. Anemics are easily recognized by a dilution of the red color. The flexure in the tail of the mutant type can usually be observed at birth, and the newborn animals with flexed tails are anemic as a rule. It was conceivable that the anemia of the flexed tailed animals was so deleterious that their death rate during the first three or four weeks was much greater than the rate for their normal siblings. In an  $F_2$  generation this differential death rate would give a marked excess of normals at the time of the final count, which was almost always between the twenty-first and twenty-eighth day after birth. This was the kind of result secured by Miss PERMAR. That hereditary anemia in mice can be a lethal agent was shown by Miss DE ABERLE (1927) who found that mice homozygous for the dominant white factor possess 25 percent as much hemoglobin and 14 percent as many red cells as normal mice, and that these anemics die within ten days after birth. Such considerations suggested that the flexed character could be the expression of a single recessive Mendelian factor, but that the deficiency of flexed animals in the  $F_2$  generation might be due to classifying a few flexed mice as normals and to a higher death rate among the flexed. The writer, therefore, undertook a more critical and extensive reinvestigation of the question (HUNT 1932).

#### FLEXED $\times$ FLEXED MATINGS

One of the most important things to discover about a mutation is whether it breeds true. Mr. RUSSELL MIXTER attempted to isolate various grades of tail flexure in strains that would propagate true to form. The details of this experiment will be discussed later in the paper. Suffice it to say at this point that seven grades were recognized, and that a line of inbreeding was started for each. In every generation animals were mated which showed the original grade used when the line was started. The inbreeding experiment revealed, also, whether flexed  $\times$  flexed crosses produced anything but flexed progeny. Forty-nine such matings yielded 688 young, of which 584 were living three to four weeks later.

Table 1 summarizes the breeding results of the flexed  $\times$  flexed crosses. Eight of these were between mice whose tails were not only flexed, but also less than half the length of the body. These are referred to as "short tailed flexed." In the remaining 41 crosses the tails were of approximately normal length. The genetic behavior of the short tailed condition will be considered after noting the results for all the crosses of flexed  $\times$  flexed. Six hundred forty-eight (94.2 percent) of all the young were flexed tailed

TABLE 1  
Types of young bred by flexed  $\times$  flexed crosses.

TYPE OF CROSS	DISTRIBUTION OF YOUNG											
	AT BIRTH				AT THE FINAL COUNTING							
	FLEXED ANEMICS	SHORT TAILED ANEMICS	DOUBT-FULLY FLEXED ANEMICS	APPARENTLY STRAIGHT ANEMICS	STRAIGHT ANEMICS	FLEXED MALES	FLEXED FEMALES	SHORT TAILED FLEXED FEMALES	SHORT TAILED FLEXED MALES	SHORT TAILED FEMALES	NORMAL FEMALES	NORMAL MALES
Flexed* $\times$ flexed	574	4	16	5	2	236	256	9	1	2	3	
Short tailed flexed $\times$ Short tailed flexed	74	13				31	14	23	8	1		
Totals	648	17	16	5	2	267	270	32	9	1	2	3

\* The tails were of normal length.



anemics on the day of birth. All the remaining 40 young were anemic, but 17 were short tailed, 16 were doubtfully flexed, 5 were apparently straight tailed, and 2 were recorded as straight tailed.

It has been mentioned that the tail may be straight and yet have stiff segments. Thus the form at birth, at which time a stiff region would be difficult or impossible to identify, is presumably not a very reliable indication as to whether the animal is genetically flexed. The condition of the tail can be more accurately determined at twenty-one to twenty-eight days, when the final counts and classifications of the young were made. At this time 537 had flexed tails of approximately normal length. The sexes approached a 1:1 ratio. There were, in addition, 32 females and 9 males with short flexed tails, 1 female with a short tail, and 2 females and 3 males with *normal* tails. Thus the flexed  $\times$  flexed crosses bred 578 flexed animals (99.0 percent) and 6 non-flexed. Practically speaking flexed breeds true, but even such a small proportion of exceptions as 1 percent should not be ignored, so Mr. MIXTER applied the breeding test to these exceptional animals.

Two of the normal males ( $\sigma^7$ 125 and  $\sigma^7$ 131) produced by the flexed  $\times$  flexed crosses were mated with flexed females. The parents of these males had relatively slight flexures (grades 1 and 2). Both were anemic at birth. Male 125 mated with flexed females 123 and 124 yielded 20 offspring, all flexed at birth. Of these 20, only one survived to twenty-one days, and at that time it was found to be flexed. Male 131 paired with flexed female 132 yielded 11 young which were flexed at birth. The four alive at twenty-one days were highly flexed.

The writer has discovered several normal tailed animals in the stock cages where the flexed strain is maintained. Presumably the parents of such animals were both flexed, for we are careful to exclude foreign stock. Five such mice were found in October 1929, 2 males and 3 females. One of these males was mated with the 3 females, producing 10 normal and 17 flexed offspring. Each female bore both types of young. If the male and the 3 females be regarded as heterozygotes carrying one gene for normal tail and one for flexed, then not many more than 7 (instead of 17) of the young should have been flexed tailed. The result is explicable, however, if we assume that the grade of flexure is determined by modifying factors. In that case the normal tailed parents would be homozygous flexed, but the modifying factors would completely suppress the development of a flexure. Segregation of these modifiers would permit the flexure to appear on some of the progeny.

One of the 10 normal young mentioned in the preceding paragraphs, a male, was mated with 2 flexed females, and 8 flexed offspring were obtained. Thus this male bred like a flexed animal.

The second normal male which was found in the flexed stock cages was mated with 2 flexed females; 17 flexed offspring were bred.

It is regrettable that only 2 of the 6 non-flexed young recorded in table 1 could be tested genetically. The exceptional normal progeny of flexed  $\times$  flexed crosses merit further study. Nevertheless Mr. MIXTER's crosses, described above, strongly favor the view that such normal young are homozygous flexed animals in which flexure, or the equivalent stiffness of the tail without flexure, is suppressed by modifying factors, or by environmental agents, or by both. The same causes which inhibit flexure may be, at least in part, responsible for the great variability in bent tails. It should be remembered in this connection that some stiff tailed animals, as has already been mentioned, approach the normal condition so closely that it is difficult to classify them. The normal offspring from flexed  $\times$  flexed matings may reasonably be regarded as completing the full range of variation for homozygous flexed tailed mice. This range extends, therefore, from marked kinking and extensive rigidity to a completely normal somatic condition.

When Mr. MIXTER's data on the offspring of flexed  $\times$  flexed crosses are combined with Miss PERMAR's, we find that such crosses bred 715 flexed and 6 non-flexed progeny. The conclusion may be drawn that the flexed tailed character breeds true as a rule, and that the few exceptional cases are conformable with the view that there is a single gene for flexed.

Mention has been made of eight crosses of short tailed flexed  $\times$  short tailed flexed. These are recorded in table 1. The tail of the short tailed mouse is less than half the length of the body. Is this short tailed condition inherited and is it caused by a gene or genes other than that for flexed?

There were 77 progeny, at the final counting, from the crosses short tailed flexed  $\times$  short tailed flexed. Of these, 45 were "long tailed" and 32 were "short tailed" ( $41.56 \pm 3.79$  percent short tailed). There were 507 young from the crosses flexed  $\times$  flexed where the tails of the parents were of normal length. Of the 507, 10 were short tailed ( $1.97 \pm .42$  percent). Thus when both parents were flexed and short tailed, the percentage of short tailed young was 21 times as great as when the flexed parents were long tailed. The difference in percentages is statistically significant, so that the short tailed condition appears to be hereditary. Short tailed  $\times$  short tailed produced both long and short tailed progeny, and the same result was obtained when long tailed flexed animals were mated together, though in the latter case the percentage of short tailed offspring was much the lower. These facts suggest that the short tailed condition is attributable to two or more pairs of genes other than that for flexed. Probably but few short tailed animals are homozygous for short tailed genes, hence when mated together they produce a considerable percentage of long tailed

mice. On the other hand when a long tailed animal carrying some of the short genes happens to mate with another long tailed individual carrying different shortening genes, a few of the progeny will be shorts. The data, however, are not sufficient to establish the mode of inheritance of the short tailed condition. Further investigation is needed. Among other things variation in the length of flexed tails should be studied to determine whether Mr. MIXTER's definition of the short tail is purely arbitrary. Also, if short tail is not due to the gene for flexed, short tailed non-flexed strains should be built up for genetic experimentation.

#### FLEXED $\times$ NORMAL MATINGS ( $P_1$ 's)

If there were a single gene for the flexed character, it remained to be seen whether it was dominant or recessive to the normal straight and pliable condition of the tail. So flexed males were bred with normal females. Thirty-nine such crosses were made, and 53 litters containing 302 animals at the time of the final counting (21 to 28 days) were produced. Of these, 299 (162 males and 137 females) had normal straight tails, while 3 mice (1 male and 2 females) possessed flexed tails. These 3 flexed animals were the progeny of one female (HY15); the remaining 38 females bred normal young only.

Female HY15 produced 14 young, 12 of which survived to the time of the final count. The distribution at birth was as follows: 3 flexed anemics, 1 doubtfully flexed anemic, 8 normally red straight tailed, and 2 anemic straight tailed. The final count of her litters after weaning was 7 normal tailed males, 2 normal tailed females, 1 flexed tailed male, and 2 flexed tailed females, or a ratio of 9 normals:3 flexed. The fact that only 1 of the 39 normal females gave birth to flexed young marks her as unusual. There was something in her germ-plasm that was absent in the other mated females. She was undoubtedly heterozygous, carrying the recessive gene for flexed and its normal dominant allelomorph. The probability that this view is correct is supported by the fact that flexure has cropped out several times in different strains of our mouse colony.

Miss DOROTHY PERMAR's thirteen matings of flexed with normal tailed animals gave 142 offspring, all of them with normal tails. Adding Miss PERMAR's data to those I secured from the 38 normal females gives a total of 432 normal tailed  $F_1$  progeny from the  $P_1$  flexed  $\times$  normal crosses. Thus flexed is recessive to the normal condition.

#### THE BACKCROSS ( $F_1 \times$ FLEXED)

The next genetic test made was to cross males and females of the  $F_1$  generation with flexed animals. The results are summarized in table 2. Fifty-one matings were made between  $F_1$  females and flexed males. They

TABLE 2  
*Distribution of the progeny of the crosses  $F_1 \times \text{flexed}$ .*

TYPE OF MATING	NUMBER OF MATINGS	NUMBER OF LITTERS	PROGENY											
			NUMBER AT BIRTH						NUMBER AT THE FINAL COUNT					
			FLEXED TAILED		NORMAL TAILED		INTERMEDIATE OR DOUBTFUL	FLEXED TAILED		NORMAL TAILED		INTERMEDIATE OR DOUBTFUL		
			ANEMIC	NORMALLY RED	ANEMIC	NORMALLY RED		♂	♀	♂	♀			
$F_1 \varnothing \times \text{Flexed } \sigma^7$	51	112	309	2	78	442	22	10	143	162	245	219	13	13
$\text{Flexed } \varnothing \times F_1 \sigma^7$	5	19	35	1	0	57	6	1	12	16	27	28	0	0
Totals	56	131	344	3	78	499	28	11	155	178	272	247	13	13
Undepleted litters from cross $F_1 \times \text{flexed}$	44	75							108	115	154	148	7	7
Undepleted litters of 7 or more animals each, from cross $F_1 \times \text{flexed}$	28	43							176		198		9	

produced 112 litters comprising 863 young, of which 795 survived to the age of 21 to 28 days, when they were counted. The reciprocal cross involved 5 matings which bred 19 litters. Combining the data for the two types of crosses, the distribution of the young at birth was as follows: 344 anemic flexed tailed; 3 normally red flexed tailed (a type of considerable significance which will be discussed later); 78 anemic normal tailed; 499 normally red normal tailed; 28 anemics and 11 normally reds whose tails were of intermediate or doubtful character.

Mention may be made at this point of the frequency of anemia among these newborn mice, though we will return to the subject later. Of the 963 young, 450, or  $46.73 \pm 1.09$  percent, were anemics. If anemia were a simple Mendelian recessive we would expect about 50 percent of the backcross generation to be anemic. The actual percentage deviates from 50 percent by  $3.27 \pm 1.09$  percent, which is on the borderline of statistical significance.

There were 386 of the newborn which were recognizably flexed tailed, intermediate, or doubtfully flexed. This was  $40.08 \pm 1.07$  percent of the whole. The appearance of a tail at birth, however, is an unreliable index to its real nature. One must wait until the skeletal elements are more fully formed before passing judgment, for, as has been pointed out, the tail of a partly grown mouse may be straight but as stiff in places as tails which show flexures. Also, a flexed tail may approach so closely to the normal structure that its nature is indicated in the mature mouse only by a very slight bend, or by a very short stiff segment, or both. Thus a more reliable classification of the tails can be made when the animals are three or four weeks old.

The final count was when the young were 21 to 28 days of age. The nature of the tail is usually evident at that time. There were 333 clearly flexed (155 males, 178 females), 26 of intermediate or doubtful nature (13 males, 13 females), and 519 normal tailed (272 males and 247 females). The very slightly flexed (intermediate) and doubtful cases have been combined with the obviously flexed throughout the paper in computing the percentages of flexed animals. The flexed condition grades into normality, so it seems reasonable to classify the few doubtful cases as extreme variants of flexed. The percentage of flexed animals at the final count was  $40.89 \pm 1.12$  percent. This is not substantially different from the proportion of flexed animals at birth, but perhaps the close agreement is merely a coincidence. A considerable number of young flexed animals may have died before the final count, the percentage being maintained by the later discovery of a sufficient number of stiff but straight tailed animals to hold up the percentage.

As the investigation progressed we began to suspect that the flexed

character is semi-lethal, so that the death rate for young flexed tailed animals would be higher than for their normal siblings. If there were a recessive flexed gene, then we would find that the percentages of flexed animals in the backcross and the  $F_2$  generations would be less than the expected 50 percent and 25 percent. Such a differential death rate could operate before birth, after birth, or during both periods. The disturbing effect of postnatal deaths can be eliminated by using only those litters which contained the same number of young at the final counting as at birth—the undepleted litters. The effect of a differential prenatal death rate on the normal:flexed ratio could be measured by determining the number of zygotes formed, then using only the litters which had been undepleted by death within the uterus. This would have involved counting the corpora lutea in the pregnant females. Since this involves a time-consuming and somewhat difficult technique we did not attempt it. However, the disturbance caused by a prenatal differential death rate can probably be reduced by using only the *large* postnatally undepleted litters, and this we did, as will appear shortly.

There were 44 matings, producing 75 undepleted litters with a total of 539 young. As stated previously an undepleted litter was one in which no deaths occurred between birth and the final counting of the litter when it was three to four weeks old. The distribution of the young in these litters is shown in table 2. If, as previously done, we class the 14 intermediate or doubtful cases with the flexed category, the percentage of the latter rises to  $43.97 \pm 1.44$  percent. Thus postnatal deaths decreased the percentage of flexed animals by about 3 percent.

The postnatal death rate among flexed animals in the 131 unselected litters was apparently rather high. 43.97 percent of the young in the undepleted litters were flexed tailed. This percentage is based upon 539 animals and is therefore quite reliable. There were 963 on the day of birth in the unselected litters. If none of these young had died, there should have been about 423 flexed animals at the final count when they were 3 or 4 weeks old ( $963 \times .4397$ ). There were, however, 359 flexed young at this time, indicating that around 64 such animals must have died. This is a death rate of about 15.13 percent. Using these data, the computed death rate among the straight tailed young of the unselected litters was 3.89 percent. Thus deaths among the flexed were nearly four times as frequent as among normals, proving our contention that there is a differential death rate in mixed litters. This matter will be considered again in connection with the  $F_2$  generation.

We used those undepleted litters which contained 7 or more animals each to eliminate as far as possible the complications arising from a prenatal, in addition to the postnatal, differential death rate. There were 43

such litters from 28 matings (table 2). These litters were probably fairly highly selected for a low prenatal death rate. The fact that they suffered from no postnatal deaths whatever suggests that they were relatively free from hereditary weaknesses, and that the mothers were in good health during gestation as well as when nursing. Such conditions should have reduced the prenatal deaths. Also, selecting litters of average size (7) and larger undoubtedly eliminated some in which a considerable proportion of the fetuses died.

If using these large undepleted litters eliminated all prenatal deaths, and if there is a single recessive flexed gene present in all the  $F_1$ 's, then these backcross litters should have contained approximately 50 percent of mutant animals. It is unlikely that there were no prenatal deaths, but they were probably few, and in this case nearly half the young should be flexed if there is a recessive gene for flexure. A hypothetical example will illustrate the point.

Suppose 2,000 zygotes are formed, half of them heterozygous for flexed and half homozygous. Assume that the prenatal death rate is low, say 5 percent. There will be 100 deaths. If, as in the first two or three weeks after birth, about four times as many flexed as normal zygotes die, then approximately 80 of these dead fetuses will be flexed and 20 normal. Thus at birth the litters will contain 920 flexed and 980 normals, and the percentage of the former will be 48.42 percent.

Let us now turn to the facts. The 43 undepleted litters containing 7 or more each produced 383 animals, of which 176 were flexed, 198 normals, and 9 had tails which were intermediate or of doubtful nature. If we include the 9 intermediate or doubtful cases with the flexed, the percentage of these in the backcross litters becomes  $48.30 \pm 1.72$  percent. This is in close agreement with what one might expect with a low prenatal death rate, considerably higher among the flexed than the normals, if there is a recessive gene for flexed.

The above is  $7.41 \pm 2.05$  percent higher than the frequency of flexed ( $40.89 \pm 1.12$  percent) in the unselected litters. This difference is 3.6 the size of its probable error and is therefore statistically significant.

The possible rôle of modifying factors should be mentioned again. Some of the variability in flexed animals is no doubt due to such genes which sometimes are so potent as to suppress the character in a homozygote. A part of our flexed deficiency in the backcross generation may be the work of these modifiers, though one would not expect them to be so numerous and their collective effect so potent in the backcross as in the  $F_2$  generation, because the backcross progeny had both a flexed parent and grandparent, while the  $F_2$  animals had only flexed grandparents.

GENETIC CONSTITUTION OF THE STRAIGHT TAILED PROGENY OF  
THE  $F_1 \times$  FLEXED CROSSES

If there is a recessive gene for flexed, then the normal young produced by the  $F_1 \times$  flexed crosses should be heterozygous for flexed, and should therefore breed like  $F_1$ 's. The constitution of these normal tailed mice can be tested by crossing them with flexed animals. If  $F$  be the symbol for the normal gene, and  $f$  for the flexed, then the following describes the process:

$P_1$  generation:  $FF \times ff$

$F_1$  generation: all  $Ff$

Backcross:  $Ff \times ff$

Progeny of the backcross:  $Ff$  (normal) +  $ff$  (flexed).

If the  $Ff$ , normal tailed, offspring of the backcross were mated, they should give practically the same results as though the  $F_1$ 's were substituted for them. Such an experiment was carried out by crossing 76 normal tailed female offspring of the  $F_1 \times$  flexed matings with flexed males. The results are summarized in table 3, where comparisons are made with the  $F_1 \times$  flexed matings. Seventy-nine litters were secured. As a rule only one litter was bred by each female. The primary object of the experiment was at first to determine whether any of these somatically straight tailed females were homozygous for the flexed factor. Therefore, as soon as a female was found to produce both normal and flexed young she was no longer used.

TABLE 3

*Comparisons between the progeny of the  $F_1 \times$  flexed crosses and the progeny of the crosses (straight tailed female offspring of  $F_1 \times$  flexed)  $\times$  flexed.*

PERCENTAGE OF:	PROGENY OF:	
	$F_1 \times$ FLEXED	(NORMAL TAILED FEMALE OFFSPRING OF $F_1 \times$ FLEXED) $\times$ FLEXED
Flexed tailed young at birth	40.08 $\pm$ 1.07	44.07 $\pm$ 1.54 (472 young)
Flexed tailed young at the final counts	40.89 $\pm$ 1.12	39.07 $\pm$ 1.67 (389 young)
Flexed tailed young in undepleted litters	43.97 $\pm$ 1.44	43.01 $\pm$ 2.45 (186 young)
Flexed tailed young in undepleted litters where the litters contained 7 or more young	48.30 $\pm$ 1.72	47.06 $\pm$ 4.08 (68 young)
Anemic young at birth	46.73 $\pm$ 1.09	44.91 $\pm$ 1.58 (452 young)
Anemic young at birth in litters of 7 or more animals	47.21 $\pm$ 1.29	45.88 $\pm$ 2.41 (194 young)

The breeding behavior of these normal tailed females closely resembled that of the  $F_1$ 's. The normal daughters of the  $F_1 \times$  flexed crosses when



mated with flexed males produced 472 young, of which 44.07 percent were recognized as flexed at birth. The corresponding figure for the progeny of  $F_1 \times$  flexed experiments was 40.08 percent. When the final counts were made at 21 to 28 days, 39.07 percent of the 389 young from the normal tailed females were found to be flexed as compared with 40.89 percent for the backcross progeny. Undepleted litters (those in which there were no deaths between birth and the final count) yielded about 43 percent of flexed animals in both experiments. Undepleted litters of 7 or more young contained 48.30 percent of flexed animals in the backcross experiment, and 47.06 percent in the other. The frequency of anemia also was about the same in the two series. The percentage of flexed animals in the progeny of the  $F_1 \times$  flexed matings is not significantly different in any of the six comparisons from the percentage of flexed among the offspring of the normal females produced by the  $F_1 \times$  flexed pairs. This is obvious when one inspects the differences and probable errors in table 3.

The normal tailed daughters of  $F_1 \times$  flexed crosses therefore breed like  $F_1$  animals, and this is exactly the result to be expected if there is a recessive flexed gene. The data of table 3 therefore furnish one more link in the chain of evidence that flexed is a simple recessive Mendelian character.

Mention has been made of the fact that the original purpose of this experiment was to determine whether somatically normal tailed animals might not be homozygous for flexed. Environmental factors in embryonic development might, conceivably, prevent permanent fusions between caudal vertebrae. If such an event occurred fairly frequently, the result would be a deficiency of flexed animals such as was found in the backcross litters. Since flexed  $\times$  flexed matings yield almost nothing but flexed young, a homozygous flexed female whose tail appeared normal might be expected to produce none but flexed tailed offspring when mated with a flexed male. As a matter of fact only one of these 72 females that gave birth to litters had only flexed young; all the remaining 71 produced both flexed and normals. The exceptional female gave birth to 6 flexed animals in one litter, 3 of which survived. She had two small litters later, but all these infants were born dead. This female may have been heterozygous for flexed rather than homozygous, for the production of 6 flexed young when a heterozygote is bred with a flexed mate should occur about once in 64 times. Somatically normal but genetically homozygous flexed mice can not be numerous enough to account for all the shortage of flexed animals in our experiments.

#### THE $F_2$ GENERATION

Miss DOROTHY PERMAR, as has been mentioned, found an  $F_2$  ratio of 7.26 normals:1.00 flexed. This ratio conforms to no simple Mendelian

mechanism of inheritance. As Miss PERMAR's work progressed several sources of error became evident which, it was thought, might account for the marked deviation from a 3.00:1.00 ratio. For example, it is probable that some straight tailed animals having stiff caudal segments were erroneously classified as normals. The practice of palpating the tail to locate stiffened sections began after the investigation was well advanced. Also, it was not realized at the outset that extreme variants of the flexed can scarcely be distinguished from normal tailed mice. This fact, too, was brought out when we began to palpate carefully all apparently normal tails in generations that produced both flexed and normal animals. So the writer repeated the experiment, starting from the beginning with fresh pairs of  $P_1$ 's.

Three flexed tailed males were mated with normal females from our laboratory stocks. Each male was confined in a cage with 6 females, so that there were 18  $P_1$  crosses in all. The  $F_1$  generation has already been discussed. The distribution of the  $F_2$  young is shown in table 4. Forty-four  $F_1$  matings bred 206  $F_2$  litters containing 1478 young on the day of birth. The condition of the tail in some of these new-born mice was, as in the previous experiments, difficult to determine, so that a considerable number were classified as "intermediate or doubtful." Whether the animal was normal blooded or anemic was likewise problematical in over 2 percent of the cases. If the intermediate or doubtful cases are combined with those that were certainly flexed, there were 289 flexed and 1189 normal tailed, a ratio of 4.11 normals:1.00 flexed, or  $19.55 \pm 70$  percent flexed. Three hundred and ten of the new-born mice were certainly anemic and 1136 clearly normal blooded, as judged by the redness of the mouse. Thus there were 3.66 normally red animals:1.00 anemic, or  $21.44 \pm .73$  percent of the young, exclusive of the doubtful cases, were anemics.

The discussion of the backcross experiments brought out the fact that the classification of tails soon after birth can not be very reliable because the only criterion for flexure at that time is external appearance. The distribution of  $F_2$ 's at the final count (when the mice were 21 to 28 days of age) is shown in table 4. We assume that the intermediate or doubtful cases were usually extreme variants of flexed in the direction of normality. Combining them with the flexed, there are 213 flexed and 1104 normal tailed animals, which make a ratio of 5.18 normals:1.00 flexed, and  $16.17 \pm .68$  percent of flexed tailed mice. This is a wide departure from the 25 percent for a Mendelian character in an  $F_2$  generation, so it is necessary to determine whether here also a higher death rate among the flexed than for the normals may have caused a deficiency of flexed.

Table 4 gives the distribution of animals in undepleted  $F_2$  litters, by which is meant those litters which suffered no mortality at all between

TABLE 4  
Distribution of the  $F_2$  generation.

NUMBER NUMBER OF F <sub>1</sub> OF F <sub>2</sub> MATINGS LITTERS		PROGENY															
		NUMBER AT BIRTH					NUMBER AT THE FINAL COUNT										
		FLEXED TAILED		NORMAL TAILED		INTERMEDIATE OR DOUBTFUL	FLEXED TAILED		NORMAL TAILED		INTERMEDIATE OR DOUBTFUL						
		CONDITION ANEMIC RED BLOOD UNCERTAIN		CONDITION NORMALLY RED BLOOD UNCERTAIN		ANEMIC RED BLOOD UNCERTAIN	CONDITION ANEMIC RED BLOOD UNCERTAIN		CONDITION NORMALLY RED BLOOD UNCERTAIN		ANEMIC RED BLOOD UNCERTAIN						
All F <sub>1</sub> 's	44	206	181	2	11	81	1097	11	48	37	10	77	109	545	559	12	15
F <sub>2</sub> 's of undepleted litters	39	114										57	70	339	314	6	9
F <sub>2</sub> 's of undepleted litters of 7 or more animals each	34	73										43	59	250	234	5	4

birth and the final counting at three to four weeks of age. Thirty-nine  $F_1$  matings produced 114  $F_2$  undepleted litters containing 795 animals. Of these, 127 were certainly flexed and 15 intermediate or doubtful, making 142 to be reckoned as flexed if we follow the plan of regarding as flexed all animals that are not clearly normal tailed. There were 653 normal mice, which gave an  $F_2$  ratio of 4.60 normals:1.00 flexed, or  $17.86 \pm .92$  percent of flexed tailed mice in the undepleted  $F_2$  generation. This was an increase of 1.69 percent over the percentage of flexed for the entire  $F_2$  generation (depleted plus undepleted litters), but the increase was not statistically significant, though it is important to note that when all possible disturbing effects of a differential postnatal death rate were removed, the percentage of  $F_2$  flexed animals moved toward 25 percent.

The attempt was then made to eliminate not only the postnatal, but at least part of the prenatal deaths as well. It will be recalled that the same object was sought in analyzing the backcross generation. Table 4 gives the types of young in undepleted litters which contained 7 or more animals each. There were 34 such  $F_1$  matings which bred 73  $F_2$  litters containing a total of 595 young. There were 102 clearly flexed tailed, and 9 intermediate or doubtful animals, making a total of 111 which we reckon as flexed, while the normal tailed numbered 484. The  $F_2$  ratio for these animals was 4.36 normals:1.00 flexed. The percentage of flexed animals rose to  $18.66 \pm 1.08$  percent, which was an increase of  $2.49 \pm 1.28$  percent over the  $16.17 \pm .68$  percent of flexed animals for the whole unselected  $F_2$  population. This difference was only 1.9 times its probable error. Even though eliminating all of the postnatal and part of the prenatal deaths did not cause a statistically significant increase in the percentage of flexed animals, yet there was an increase of noteworthy size, and to this extent the facts lend support to the view that a differential death rate is one of the causes for the frequency of flexed animals being considerably below 25 percent.

The probable effects of the differential death rate are shown by the following computations. There were 17.86 percent of flexed animals in the undepleted  $F_2$  litters at the final count. It is possible that if all the 1478  $F_2$  young, that is all the mice born in the  $F_2$  generation, had survived to the age at which the animals were finally counted (3 to 4 weeks), about the same percentage of flexed mice would have been found among them. In other words there would have been approximately 264 ( $1478 \times .1786$ ) flexed tailed individuals. There were actually only 213, so that something like 51 flexed animals ( $264 - 213$ ) probably died. This was a death rate of 19.32 percent for the young flexed animals. If there were 264 flexed young among the newborn, then there were 1214 normal tailed animals at that time. This number had fallen to 1104 when the young were 21 to 28 days

old, a decrease by death of 110 animals, or a death rate of 9.06 percent. Thus it appears plausible that the mortality rate was about twice as great among the animals with the defective tails as among the normals.

Possibly the undepleted litters were selected to some extent for a low percentage of flexed animals. If flexed animals have a higher death rate than normals, then there is more likelihood of deaths occurring in litters having a relatively large number of flexed animals than in litters having fewer flexed. Thus it may be that if no deaths had occurred among our whole  $F_2$  population, we would have found considerably more than 17.86 percent of flexed mice at the final count.

The data suggest modifying factors. When death rates were materially reduced the percentage of flexed animals in the  $F_2$  generation fell below 25 percent by 6.34 percent, while in the backcross generation (table 3) the percentage of flexed was only 1.70 percent less than the expected 50 percent, and among the progeny of matings between flexed males and normal tailed daughters of the  $F_1 \times$  flexed cross the percentage of flexed was only 2.94 percent less than 50 percent. The hypothesis of modifying factors fits the facts fairly well. The  $F_2$  flexed animals all had 2 flexed grandparents and 4 flexed great-grandparents. The progeny of the  $F_1 \times$  flexed crosses had a flexed parent, 3 flexed grandparents, and 6 flexed great-grandparents. The young of the matings between flexed males and normal daughters of the  $F_1 \times$  flexed crosses each had one flexed parent, 3 flexed grandparents, and 6 flexed great-grandparents. A greater concentration of flexed tailed germ-plasm is thus associated with a closer approximation to the percentages one should get if flexed is a simple recessive. This fact supports our theory that flexed is such a character, but that other genes, which are few or absent in decidedly flexed animals, may modify the character, or even suppress it altogether if enough of them are present. The conception of modifying factors finds further support in the fact that flexed is a highly variable trait which ranges all the way from very extreme forms to normality itself.

The reader has probably noted the deficiency of males among the flexed animals recorded in the tables. The progeny of the following crosses were classified by sex and condition of the tail to compare the sex ratios among flexed and normals: flexed  $\times$  flexed; flexed  $\times$  normal;  $F_1 \times$  flexed;  $F_1 \times F_1$ ; and normal female (bred by the cross  $F_1 \times$  flexed)  $\times$  flexed male. These crosses produced a total of 585 flexed males, 656 flexed females, 1097 normal males, and 1062 normal females. Thus  $47.14 \pm .96$  percent of the flexed animals were males, while among the normal mice the percentage was  $50.81 \pm .73$  percent. There was thus a slight deficiency of males in the flexed category. The difference,  $3.67 \pm 1.21$  percent, scarcely exceeded three times its probable error, so that it was of doubtful significance. However,

the males were decidedly in the minority among the flexed animals bred by three of the four crosses mentioned above, so there are grounds for suspecting that there is some factor (or factors) among flexed mice that modifies the sex ratio and that such an influence is not found among normal animals. Perhaps flexed males have a higher death rate than flexed females.

#### INHERITANCE OF THE GRADE OF FLEXURE

The fact has been emphasized that the flexed character is highly variable. There are spirals, tails with one or more angles of varying magnitude, straight tails with stiff segments, and finally occasional individuals that appear normal. Variability is of the continuous type. It was shown earlier in the paper that abnormal shortness of the tail in flexed mice is probably inherited. If this modification is determined by genes other than the flexed gene, the suspicion is aroused that perhaps all variations in flexure are more or less hereditary. This possibility was tested experimentally.

Before such a study could be undertaken it was necessary to devise a system for classifying the degrees of flexure. Since variability is strictly continuous and sharp natural boundaries between different grades do not exist, the system was necessarily somewhat arbitrary. Three fundamental characteristics could be used: (1) length, (2) the percentage of the length which was rigid, and (3) the number and magnitude of the angles. The short tailed condition has been shown to be probably hereditary, so it need not be considered further. The system of grading adopted was based on the number and size of the angles, and upon stiffness where angularity was absent or slight. Other, perhaps equally valid, criteria could have been used.

The scheme for defining the different grades was as follows:

Grade 1. No, or almost no, visible flexure. Stiffness might easily be detected in the tail when it was bent a little and passed between the thumb and index finger, or the stiffness might be slight. Doubtful cases were included in this group, that is those in which flexure, or stiffness, (or both) was so slight that it was difficult to classify them with certainty either as normal or flexed animals.

Grade 2. Characterized by one slight but well defined flexure in the tail.

Grade 3. Two or more slight flexures.

(A "slight" flexure was arbitrarily defined as one at which the distal segment was deflected by  $30^\circ$  or less from the axis of the adjoining proximal segment. If this angle was greater than  $30^\circ$  the flexure was "pronounced".)

Grade 4. One pronounced flexure.

Grade 5. Two or more pronounced flexures.

Grade 6. The flexure assumed the form of a spiral—a “corkscrew tail.”

If the type or degree of flexure is inherited, that is, if there are factors other than the flexed gene itself which determine the form of the tail, then one might expect the following: (1) The flexed animals in the  $F_2$  generation from a  $P_1$  cross of flexed  $\times$  normal might have tails resembling that of the flexed  $P_1$  ancestor; and the same thing could be true of the flexed individuals produced by a cross between  $F_1$ 's and a flexed, providing the flexed parent and grandparent were of the same, or nearly the same, grade of flexure. It is conceivable, of course, that in such experiments genes from the normal  $P_1$ 's might neutralize the effects of specific modifiers from the flexed ancestry. (2) If close inbreeding were carried on within each of the grades of flexure defined above, strains should be secured which would breed approximately true for the grade in question. (3) Finally, there should be a positive correlation between the grades of parents and offspring.

Each of these three tests for modifiers was applied. The  $F_2$  flexed animals derived from the  $P_1$  crosses flexed  $\sigma \times$  normal  $\varphi$  were graded by the system for tail classification just outlined. There were 207  $F_2$  flexed mice which descended from three mutant  $P_1$  males, all of grade 5. The distribution of these flexed  $F_2$ 's was as follows:

GRADE OF FLEXURE	1	2	3	4	5	6
Number of $F_2$ flexed mice	34	37	29	55	41	11

The above data seem to indicate that the degree of flexure is inherited, for the mode was at grade 4, and the second largest class was grade 5, while the flexed grandfathers were of grade 5. Such a conclusion is not to be drawn, however, without further investigation, for as will be shown shortly by the results of breeding mutants with one another, the modal grade among the offspring was 5, whether the parents showed a high or a low grade of flexion. Thus  $P_1$  parents of grades 2 or 3 might have had  $F_2$  descendants whose mode was at 4 or 5. An explanation will be offered later for this peculiarity in the inheritance of flexed tail.

The effect of the flexed ancestry was then determined on the grade of the backcross progeny from matings between  $F_1$  females and flexed males. Since only those matings were used in which the same flexed male was both father and grandfather of the litters produced, or the father and grandfather were of the same grade of flexure, the number of young available was limited to 161. The data are shown in table 5.

TABLE 5

FLEXED MALE ANCESTORS		GRADES OF BACKCROSS FLEXED OFFSPRING						MEAN GRADES OF OFFSPRING
FATHER	GRANDFATHER	1	2	3	4	5	6	
		NUMBERS OF OFFSPRING						
6	6 (grade 3)	9	8	7	20	30	8	3.95
5	5 (grade 4+)	1			3			3.25
1	1 (grade 5)	2	1	3	5	8		3.84
26 (grade 4)	5 (grade 4+)	1	2	2	6	2	2	3.80
27 (grade 5)	7 (grade 5)	2	2	5	15	13	4	4.15

Mean grade of offspring where the flexed ancestor ( $\sigma^6$ ) was of grade 3: 3.95

Mean grade of offspring where the flexed ancestors ( $\sigma^5$  and  $\sigma^{26}$ ) were of grade 4 or 4+: 3.68

Mean grade of offspring where the flexed ancestors ( $\sigma^1$ ,  $\sigma^7$ , and  $\sigma^{27}$ ) were of grade 5: 4.05

It is obvious that table 5 furnishes no satisfactory evidence that the grade of flexure is influenced by genes, though the normal blood in these flexed animals may have prevented such factors from manifesting themselves.

Close inbreeding was finally used to determine whether the grade of flexure was inherited. Mr. RUSSELL MIXTER collected all the data in this part of the investigation. His inbreeding experiments began late in June 1929, and continued until July 1930. If genetic factors determine the extent of flexion, then by mating animals of about the same grade, selecting siblings of this grade from among their progeny and mating them together, and continuing this process for several generations, one should be able, in some lines at least, to evolve a strain of flexed mice which varies within relatively narrow limits around the grade selected in each generation. This would be due, of course, to increasing homozygosity.

Mr. MIXTER started this experiment with 32 females, representing all the grades of flexure which have been described, each female being mated with a male of her own kind. Table 6 shows the numbers of animals,

TABLE 6

*Numbers of individuals mated in successive generations of the inbreeding experiment.*

GENERATIONS	GRADES OF INDIVIDUALS MATED						SHORT TAILED
	1	2	3	4	5	6	
First	1♂, 5♀	1♂, 2♀	1♂, 5♀	1♂, 5♀	1♂, 5♀	1♂, 5♀	1♂, 5♀
Second	1♂, 2♀	1♂, 1♀	3♂, 4♀	4♂, 7♀	7♂, 15♀	2♂, 3♀	2♂, 4♀
Third	2♂, 2♀				6♂, 10♀		2♂, 3♀
Fourth							1♂, 1♀

classified by grades, used in the first and succeeding generations. Five females having a tail flexure of grade 1 were mated with a single male



of the same grade. The matings for grades 3, 4, 5, 6, and the short tailed type each consisted, likewise, of 5 females and 1 male. Two grade 2 females were bred with a single male of that type. Thus there were 32 crosses in the first generation. It has been emphasized that the classification of the grades of flexure is arbitrary, that these grades are not separated from one another by natural gaps, but that variability is continuous. Perhaps the short tailed type shows fewer intergrades than any of the others. Mr. MIXTER defines it as having a tail which is less than half as long as the body.

The mating cages of the inbreeding experiment were inspected twice a week to discover pregnant females, which were isolated. The litters were observed every three days to make note of deaths. The young animals were weaned and usually classified when 21 to 25 days old, and one or two drawings of each tail were recorded. From among the offspring, mice of the same grade of flexure as the parents were chosen to breed a new generation. The members of the first generation were selected from our stock cages, and were but remotely, if at all, related, but in later generations the matings were always between siblings.

Mr. MIXTER was unable to develop pure lines of flexed animals because breeding practically ceased in all seven lines in the second or third generation. Several difficulties were encountered. Sometimes he obtained males whose tail structure made them eligible for breeding, but no sisters of the same grade were born, and *vice versa*. Our laboratory stock of flexed animals seemed, on the whole, to propagate less rapidly than normal mice, so only what appeared to be healthy animals were selected for inbreeding, and for this reason fewer matings than possible were made. The experiment automatically terminated at the third generation, for the third generation animals practically ceased breeding. Whether this was due to increasing sterility, to miscarriages, or to the females' inability to bear young as a consequence of malformations of the pelvic girdle, we do not know. The harmful effects of this limited course of inbreeding, combined with the inferior reproductivity of the flexed stock, are probably sufficient to account for this result. The inbreeding must have been an important factor in suppressing reproduction, for only one of the 32 females of the first generation was sterile.

In spite of the fact that the inbreeding experiment failed to establish homogeneous lines of mice, it showed that there are probably genes which cause variation in the degree of flexure. This is brought out by inspection of the distribution of young from the different types of crosses and by the correlation between parents and offspring. The matings used in MIXTER's experiment were obviously superior to the  $F_2$  and backcross material for determining whether the degree of flexure is inherited. When a flexed ani-

mal is mated with a normal one, the  $F_2$  flexed descendants might conceivably receive modifying genes not only from the flexed  $P_1$  ancestor, but from the normal one also. The genes from the latter might partly or completely neutralize the effects of modifiers from the flexed  $P_1$ , so that the resemblance between the  $P_1$  and  $F_2$  flexed mice would be very much reduced. The same criticism would apply to a lesser extent if a correlation be sought between the flexed animals of a backcross generation (produced by  $F_1 \times$  flexed matings) and their flexed ancestors.

Table 7 is a summary of Mr. MIXTER's inbreeding work. The left half of the table shows the results of mating the first generation animals, which were selected from the stock cages. The right half records the consequences of inbreeding the second generation. Table 7 reveals some very interesting facts. No grade of flexure bred true; in fact if the offspring of the first and second generation crosses are combined, it is found that in four of the seven types of matings (grades 1, 2, 4, and 5) the progeny included all grades of tail flexure. Five hundred and sixteen animals constituted the second and third generations, and of these, 248 (48.1 percent) were classified in grade 5. This conspicuous placement of the mode at 5 was found among the offspring of all the types of crosses where the numbers were fairly large. The heaping up of nearly half the young in one grade suggests that it may have included more genotypes than any of the others, and that more than one grade might well have been created from grade 5.

The relatively high percentage of short tailed progeny from crosses of short tailed animals is conspicuous. The short tailed crosses listed in table 7 produced 58 young, 23 of which were short tailed (39.7 percent). Among the 458 young bred by all the other crosses, only 16 (3.5 percent) were short tailed, and 12 of these were the offspring of the grade 5 cross. As has been previously stated, the evidence indicates that the short tailed condition is inherited.

Tables 8 and 9, particularly the latter, indicate that hereditary factors are involved in determining the extent of flexure. Table 8 shows the mean grades of the inbred flexed offspring produced by different grades of parents, the short tailed parents and their progeny being excluded. Parents of grade 1 bred 74 individuals having an average grade of 4.01. The rating of the offspring rose slightly and irregularly as the level of the parents increased to 6.

More striking evidence of factors modifying the degree of flexure is found in table 9, where parents and offspring are classified as "low grade" (grades 1-3) and "high grade" (grades 4-6).  $84.9 \pm 1.5$  percent of the offspring of "high grade" parents were themselves "high grade," while only  $65.2 \pm 2.4$  percent of the young of "low grade" parents were "high grade." The "high grade" parents thus produced 19.7 percent more "high grade"

TABLE 7  
*Distribution of offspring in the inbreeding experiment.*

GRADES OF THE FIRST GENERATION	GRADES OF THE OFFSPRING OF THE FIRST GENERATION (SECOND GENERATION)							GRADES OF THE SECOND GENERATION WHICH WERE USED AS PARENTS		GRADES OF THE OFFSPRING OF THE SECOND GENERATION (THIRD GENERATION)						
	1	2	3	4	5	6	SHORT	TOTAL	1	2	3	4	5	6	SHORT	TOTAL
1	7	3	8	9	37	4	1	69	1	2	1	2	1			6
2	4	7	7	3	21	1	1	44	2		1	1	2		1	5
3	3	2	17	11	25	2		60	3				2	1		3
4	4	2	10	16	42	11	1	86	4			1	3			4
5		1	6	14	49	9	11	90	5	1	1	4	12	28	4	51
6		2	8	4	15	11		40	6							0
Short			3	5	14		11	33	Short		1	1	1	9	1	25
Total	18	17	59	62	203	38	25	422	Total	3	3	8	15	45	6	94

progeny than did "low grade" parents, and this difference can scarcely have been due to random sampling, for it was seven times as large as its probable error. Thus it is evident that though the more extreme degrees of flexure did not breed true, yet they produced a higher percentage of markedly flexed offspring than the less pronouncedly flexed parents.

TABLE 8

*Average grades of the inbred flexed tailed offspring of the second and third generations produced by the different grades of parents. The short tailed parents and offspring are excluded.*

GRADE OF PARENTS	AVERAGE GRADE OF OFFSPRING
1	4.01 (74 individuals)
2	3.81 (47 individuals)
3	4.05 (63 individuals)
4	4.46 (89 individuals)
5	4.67 (129 individuals)
6	4.63 (40 individuals)

TABLE 9

*Distribution of offspring in relation to the high or low grade of the parents.*

GRADES OF THE PARENTS OF THE SECOND AND THIRD GENERATIONS		GRADES OF THE SECOND AND THIRD GENERATIONS			
		LOW GRADE (1-3)		HIGH GRADE (4-6)	
		NUMBER	PERCENTAGE	NUMBER	PERCENTAGE
Low grade crosses	1	23	31.1	51	68.9
	2	19	40.4	28	59.6
	3	22	34.9	41	65.1
High grade crosses	4	16	18.0	73	82.0
	5	13	10.1	116	89.9
	6	10	25.0	30	75.0
Totals		103		339	
Percentage of high grade offspring from high grade parents:				84.9 $\pm$ 1.5 percent	
Percentage of high grade offspring from low grade parents:				65.2 $\pm$ 2.4 percent	
Difference:				19.7 $\pm$ 2.8 percent	

Further evidence that the type of flexure is to some extent determined by hereditary is found in the data of table 10 where the grades of the parents are correlated with those of the offspring. These statistics too were derived from MIXTER's experiment on inbreeding. There were 39 matings which produced 487 young. In 31 of the matings both parents had the same grade of flexure; in 8 (21 percent) the two parents were of different grades, 5 pairs differing by one (2 and 1; 2 and 3; 3 and 4; 4 and 5), and 3

pairs by two grades (2 and 4; 3 and 5). Whenever the two parents were of different grades, the average of their ratings was used in table 10. This accounts for the parental grades  $1\frac{1}{2}$ ,  $2\frac{1}{2}$ ,  $3\frac{1}{2}$ ,  $4\frac{1}{2}$ , and  $5\frac{1}{2}$ . Seventy-nine (16 percent) of the young were the progeny of such crosses. The coefficient of correlation between parents and offspring was  $+.243 \pm .029$ , a rather low, but nevertheless statistically significant correlation.

TABLE 10  
*Correlation between the grades of flexure of parents and offspring.*

	GRADES OF FLEXURE OF OFFSPRING					
	1	2	3	4	5	6
6		2	8	4	15	11
$5\frac{1}{2}$						
5	3	3	12	28	97	17
$4\frac{1}{2}$	1	1	2	10	21	8
4	6	2	10	19	30	6
$3\frac{1}{2}$					2	1
3	1	2	12	9	30	
$2\frac{1}{2}$			1	1	2	
2	2	4	4	2	9	1
$1\frac{1}{2}$	1	1	3	9	15	
1	10	6	7	5	27	4

$n=487$ ;  $r=+.243 \pm .029$ ; ratio=8.4.

The mean was calculated for the parents of each grade of offspring. These six averages when plotted were found to be approximately linear, so that the coefficient of correlation may properly be used to measure the resemblance between parents and offspring. However, only about 6 percent (the square of the coefficient of correlation,  $.243^2 \times 100$ ) of the variability among the offspring can be definitely attributed to the parents. This coefficient of correlation should not be regarded as an exact measure of hereditary influences modifying the expression of flexure, for another system of classifying the tails (by the number of fused vertebrae, for example) might have given a considerably different value for  $r$ . Assuming that the stiffened condition of the tail is due to the fusion of vertebrae, it is reasonable to suppose that movements of the fetus and uterus would at critical periods in the development of the character modify the angle of flexure or break up incipient adhesions between the vertebrae. Events following birth might have similar results. Thus the form of a flexed tail is probably the resultant both of hereditary and environmental influences.

#### LINKAGE RELATIONS OF THE FLEXED GENE

Our observations indicate that there is no linkage between flexed and albinism, but data are not available for testing linkage with other char-

acters. The backcross generation was available for this study. Two types of backcrosses were made: first, the flexed albino male  $\times$  the  $F_1$  colored female (produced by the  $P_1$  cross flexed albino male  $\times$  normal tailed colored female), and second, the reciprocal of the above cross. The progeny of the cross flexed albino male  $\times$   $F_1$  colored female were as follows:

Flexed albinos:	108	Flexed colored:	106
Intermediate or doubtful tailed, albinos:	<u>9</u>	Intermediate or doubtful tailed, colored:	<u>9</u>
Total counted as flexed albinos:	117	Total counted as flexed colored:	115
Normal tailed albinos:	172	Normal tailed colored:	177

The "intermediate or doubtful tailed" were, as has been mentioned, those cases where the presence of a very slight flexure or an indistinct stiffness in the tail justified the view that they could not be normals. We have assumed that such mice were extreme variants in the flexed distribution.

The analysis of the data is complicated by the differential death rates which seemed to be higher among the flexed than the normals in these mixed litters, and by such extreme variability of the flexed that some of them looked like normals. So for clarity it may be well to set down the genetic formulae.

$F$  = gene for normal tail.

$f$  = gene for flexed tail.

$C$  = gene for color.

$c$  = gene for albinism.

$P_1$  zygotes:  $ffcc$  male  $\times$   $FFCC$  female

$P_1$  gametes:  $fc$   $FC$

$F_1$  females:  $fc \cdot FC$

$F_1$  ova :  $fc + FC + fC + Fc$

When such  $F_1$  females are mated with flexed albino males, the backcross progeny should be,

$ffcc$	+	$FfCc$	+	$ffCc$	+	$Ffcc$
(flexed albinos)		(normal tailed colored)		(flexed colored; crossovers)		(normal tailed albinos; crossovers)

The deficiency of flexed animals in the backcross generation was, as has been repeatedly asserted, probably due to a higher death rate among the flexed, and to flexed individuals that looked like normals. The overlapping can not have been very extensive in this particular backcross population, because among the large undepleted litters, where all the postnatal and

part of the prenatal deaths had been eliminated, 48.3 percent of the young were flexed.

The crossover percentage may be computed in various ways. What percentage of the  $F_1$  flexed bearing germ cells were of the crossover type ( $fC$ )? The death rate among the  $ffcc$  young was probably about the same as among the crossover class,  $ffCc$ . The percentage of flexed albino young ( $ffcc$ ) which were overlaps (that is, seemed to be normal tailed albinos, ( $Ffcc$ ) was probably about equal to the percentage of flexed colored young ( $ffCc$ ) which likewise were overlaps (looking like the normal tailed colored,  $FfCc$ ). Thus the agencies which decreased the  $ffcc$  class undoubtedly operated with the same intensity on the  $ffCc$  crossover group, so that the percentage of flexed backcross young which had colored hair was probably about the same as the percentage of flexed bearing  $F_1$  germ cells which carried the color factor. Thus computed, the percentage of crossing over was  $49.57 \pm 2.21$  percent, which does not differ significantly from the 50 percent to be expected if no linkage exists.

In similar fashion one may determine what percentage of the  $F_1$  germ cells that carried the gene  $F$  for normal tail, also bore the factor for albinism  $c$ . This becomes a matter of determining what percentage of the normal tailed young were albinos ( $Ffcc$ ). As just pointed out, each of the two normal tailed classes no doubt contained a few flexed overlaps, but it has also been shown that they were probably not numerous in either

case. The crossover percentage for the  $F$  bearing ova would be: 
$$\frac{172}{172+177} \times 100 = 49.28 \pm 1.81 \text{ percent.}$$

If we use all four classes of backcross young to compute the percentage of crossing over by the conventional method, we get  $49.40 \pm 1.40$  percent.

A limited amount of data (83 animals) was secured from the reciprocal cross,  $F_1$  male (produced by the  $P_1$  cross flexed albino male  $\times$  normal tailed colored female)  $\times$  flexed albino female. All four classes were used to compute the crossover percentage, which was found to be 51.81 percent. Thus albinism and the flexed gene are not located on the same chromosome, which means, also, that flexed is not linked with the genes known to be linked with, or allelomorphous to, albinism (full color, chinchilla, extreme dilution, dark-eye, pink-eye, non-shaker, shaker).

Inspection of the data presented in the tables shows that the flexed character is not sex-linked.

#### SUMMARY

1. The flexed tailed mutation was discovered in a stock of normal mice. It is characterized by stiff angular bends or spirals, or by rigid segments without flexures.

2. The character is highly variable, some tails being extremely bent in one or more places, while a few homozygous flexed animals can be distinguished from normals only by breeding tests.

3. The progeny of flexed  $\times$  flexed crosses are nearly all flexed, the few exceptions being animals whose tails are normal but which are probably all homozygous flexed. There is evidence that the flexed character may be suppressed by other factors.

4. Short tail is an hereditary variation, found in the flexed population, which is probably due to several genes.

5. The flexed tailed character is recessive.

6. The percentage of flexed young produced by the backcross ( $F_1 \times$  flexed) approaches 50 percent when the postnatal and part of the prenatal deaths are eliminated from the data.

7. The normal tailed female offspring of  $F_1 \times$  flexed matings have the same genetic constitution as  $F_1$ 's.

8. The percentage of flexed animals in the  $F_2$  generation is in agreement with the views that flexed is a recessive character which can be suppressed by modifying genes, and that the death rate for flexed mice is higher than for normals.

9. The coefficient of correlation between the grades of flexure in flexed parents and their offspring is  $+ .243 \pm .029$ , indicating that the grade of flexure is to some extent inherited.

10. The flexed gene is not linked with albinism, and it is not sex-linked.

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# ANEMIA IN THE FLEXED TAILED MOUSE, *MUS MUSCULUS*

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HUNT, MIXTER, and PERMAR (1933) have described the mode of inheritance of flexed tail in the house mouse, a mutation discovered by HUNT in the rodent colony of the Department of Zoology at the MICHIGAN STATE COLLEGE. Flexed tailed mice are characterized by fusions between adjacent caudal vertebrae, and as a rule by angular or spiral bends in the tail. The character is a Mendelian recessive, but there is a deficiency of flexed individuals in  $F_2$  and backcross populations because the mutants have a higher death rate than their normal siblings. Newborn flexed mice are anemic, and the purpose of this paper is to describe the inheritance and characteristics of this anemia.

To avoid the reprinting of tabular data from the first paper, the tables of the two publications are numbered as one series, tables 1 to 10 being in the first, and 11 to 17 in this contribution.

## THE INHERITANCE OF ANEMIA

Early in the experiments the writer (H. R. HUNT) noticed that in litters bred by matings between flexed and  $F_1$  animals, the flexed young were considerably lighter in color than their normal tailed siblings. The hairless condition of newborn mice gives them a reddish color, for the blood shows through the skin. The lighter pink of the flexed mouse immediately after birth is frequently more noticeable on some parts of the body than others. The hips and top of the head are particularly favorable places for detecting the paleness. It seemed to the writer that the lightness of the flexed young was probably due to an anemia of some kind, so Mr. RUSSELL MIXTER undertook a study of the numbers of erythrocytes and the hemoglobin contents of blood from flexed and normal tailed animals at different ages.

The anemic appearance of young flexed mice varies, in some animals the condition being very obvious, while in others careful scrutiny is necessary to detect any difference from the normal siblings. The litters should be examined for classification on the day of birth and by good daylight. Occasionally one can not decide definitely whether the young mouse is anemic or normal, and such cases have been designated as doubtful. Flexed young are usually anemic at birth, but straight tailed newborn mice frequently show this condition, and on the other hand a flexed ani-

mal is occasionally as red as its normal newborn siblings. These matters will be considered when discussing whether the flexed and anemic conditions are due to the same gene.

This section is intended to throw light on the mode of inheritance of anemia, which was discovered only after the work on the inheritance of flexed tail was under way. Thus the experiments were not designed for its study, so they did not definitely establish the way in which it is inherited. For example, we do not know whether this anemia breeds true, nor are we certain that our  $P_1$  flexed animals were anemic at birth. However the writer's experiments accumulated considerable data all of which are consistent with the view that anemia, like flexed, is a Mendelian recessive, so the facts are presented as a contribution to the question and not as a final solution.

Table 11 summarizes the evidence concerning the inheritance of anemia. It includes the  $F_2$  generation, the offspring of  $F_1 \times$  flexed matings, and the progeny of flexed males paired with normal females which, in turn, were bred by crosses between  $F_1$  and flexed animals. Unfortunately no special effort was made to study anemia in the  $F_1$  generation, but the records show that four litters containing 20 animals were observed, and that all were normal.

The  $F_2$  generation will be considered first. Every doubtful case was excluded from the  $F_2$  data presented in table 11. All the dead have been eliminated, for the identification of a dead individual as anemic or normal is hazardous. Also, if the classification of one or more animals in a litter was uncertain, the whole litter was excluded from the table. The small number of such doubtful cases may actually have been selected at random from the normal and anemic classes, but there is ground for the suspicion that they had mild cases of anemia. To have eliminated such cases without disposing of their normal siblings would have unjustifiably reduced the percentage of anemics. There were 1373  $F_2$ 's, of which 1076 were normal and 297 clearly anemic. Thus  $21.63 \pm .75$  percent were anemic.

This is 3.37 percent less than the 25 percent expected on the assumption that anemia is recessive, yet it approximates 25 percent closely enough to arouse the suspicion that anemia is actually such a character. It has already been shown that flexed animals probably have a higher prenatal death rate than normals, so the deficiency in anemic young may be due to the same cause. This difficulty may be partly overcome by considering only large litters. In table 11 the data are given for litters of 7 or more, and 9 or more young. A population consisting of litters of nine or over on the day of birth must have been selected very strongly against a prenatal death rate, and therefore against any differential rate as between normals and anemics. This technique has been discussed already in con-

TABLE 11  
*The inheritance of anemia.*

CLASSES OF NEWBORN MICE	FREQUENCY OF ANEMIA AMONG NEWBORN MICE							
	IN ALL LITTERS		IN LITTERS OF 7 OR MORE YOUNG				IN LITTERS OF 8 OR MORE YOUNG	
	NORMALS	ANEMICS	PERCENT ANEMIC	NORMALS	ANEMICS	PERCENT ANEMIC	NORMALS	PERCENT ANEMIC
F <sub>2</sub> generation	1076	297	21.63 ± .75	755	216	22.25 ± .90	363	108 22.93 ± 1.31
Progeny of F <sub>1</sub> × flexed	490	424	46.39 ± 1.11	360	222	47.21 ± 1.29		
Progeny of normal females (bred by the cross F <sub>1</sub> × flexed) × flexed males	241	195	44.72 ± 1.61	105	89	45.88 ± 2.41		

nection with the inheritance of flexed (HUNT, MIXTER, and PERMAR 1933). There were 971 young in litters of 7 or more per litter. Of these, 216 were anemic, or  $22.25 \pm .90$  percent. Litters including dead animals were excluded, not only on account of the possibility of classifying the dead erroneously, but also because death may have occurred before birth, and the aim was to reduce the effect of the prenatal death rate as much as possible. Where the condition of the blood was doubtful in one or more animals, the whole litter was thrown out of the computation. If from the above litters of 7 or more young, those containing 9 and over are considered, there were 108 anemics and 363 normals, or  $22.93 \pm 1.31$  percent of anemics. Partial correction for a prenatal mortality thus raises the percentage of flexed animals to within about 2 percent of the 25 percent that should be approximated if anemia is due to a single recessive factor. This justifies the suspicion that the type of anemia with which we are dealing may be caused by such a gene.

The fact should not be overlooked, however, that to secure even such a high percentage of anemic young as 22.93 percent in  $F_2$ , the prenatal death rate among the anemic young might be quite high. The following computations illustrate this point, assuming that anemia is a simple recessive character.

Assumed prenatal death rates of normals.	Computed prenatal death rates of anemics.
0 percent	10.7 percent
3 percent	13.6 percent
5 percent	15.0 percent

The prenatal death rates would have to be not far from 10 percent higher among anemics than normals, even in the litters of 9 or more, to give 22.93 percent of anemics. The anemia may, conceivably, so handicap the fetus that such a differential mortality actually exists. A conclusive study of prenatal death rates, using comparable normals as controls, is highly desirable.

Table 11 shows the results of backcrossing  $F_1$ 's with flexed animals. Let us first consider all litters, regardless of their sizes. Here again all dead young were discarded in the computations, and in addition all litters were eliminated which contained one or more animals whose blood status was doubtful. The reasons for these omissions have already been discussed in connection with the  $F_2$  generation. There were 914 young mice of which  $46.39 \pm 1.11$  percent were anemic. This is 3.61 percent less than the 50 percent to be expected in a backcross generation if the character is a simple Mendelian recessive. The table gives the data, also, for litters of 7 or more. Litters containing dead young, or young which could not with certainty

be classified either as normal or anemic, have been omitted. This selection of litters on the basis of large size would be expected to increase the percentage of anemic animals if they have more than the normal prenatal mortality.  $47.21 \pm 1.29$  percent were anemic, which was a small and statistically unreliable increase over  $46.39 \pm 1.11$  percent. Again the facts, as far as they go, justify the idea that the anemia may be the expression of a recessive gene, for the percentages are suspiciously close to 50 percent.

The last horizontal column of table 11 deals with the results of crossing flexed males with normal females which were bred by crosses of  $F_1$  with flexed animals. The litters used in computing the percentages were selected in exactly the same ways as in the two preceding experiments. If the anemia is due to the flexed gene itself, or to a recessive gene very closely linked with flexed, then one would expect an approximation to 50 percent among the offspring from such matings. The percentages of anemics actually found were  $44.72 \pm 1.61$  percent for all the litters, and  $45.88 \pm 2.41$  percent in litters of 7 or more. These percentages, again, are suggestive.

#### STUDY OF THE BLOOD OF THE FLEXED TAILED MOUSE

Mr. RUSSELL MIXTER was assigned the task of determining the numbers of erythrocytes and leucocytes in the bloods of flexed and normal mice at birth and subsequent ages, and of measuring the hemoglobin percentages in the same strains. These studies, together with the material on inbreeding already reported, were submitted as a graduate thesis in the Department of Zoology at the MICHIGAN STATE COLLEGE. No attempt will be made to review the voluminous literature on anemia in man and animals, but Mr. MIXTER's findings will be presented together with some comments on the genetic significance of his observations.

It was necessary, of course, to compare the cell counts and hemoglobin percentages of flexed mice with corresponding data from normal controls to determine whether the flexed animals were actually anemic. Two types of controls were used: normals carrying flexed (heterozygotes), and homozygous normals. Flexed and heterozygous normal tailed young were secured by mating heterozygotes with flexed. Seven heterozygous ( $F_1$ ) normal males were mated with 14 flexed females. In addition, 1 flexed male was bred with 6 heterozygous normal females. The mixed litters produced by these matings provided material for reliable comparisons between normal and flexed young. As a rule, when observations were made on a flexed animal the same procedure was carried out on a normal litter mate. Thus such factors as health of the mother, temperature of the animal house, chance fluctuations in the quality of the food, external and internal parasites, et cetera, which may have affected the erythrocyte and hemoglobin content of the flexed animals, would produce, presumably, similar effects

on the heterozygous controls, so that the differences between the groups were due in the main to the normal gene of the heterozygous controls.

The other type of control was the homozygous normal. These mice were produced by 1 homozygous normal male mated with 8 homozygous normal tailed females. The offspring of these crosses lived under approximately the same conditions as the flexed animals, so differences between them could not have been due, in any considerable degree, to the environment. But the heterozygous siblings of the flexed young were doubtless better controls than the unrelated homozygotes, because the former more than the latter must have resembled the flexed young in the distribution of genes other than the flexed gene. It is important to remember these distinctions, for, as will appear later, the two control series differed from one another as well as from the flexed mice.

Blood cell counts and hemoglobin determinations were made at birth, at one, two, three, and four weeks after birth, and further at six, eight, ten, and twelve weeks. Adults also were investigated. These were mainly the parents of the young on which observations were made. Four of the flexed adults were not parents of the mice used in the blood problem, but of those recorded in the inbreeding experiment. The ages of the adults ranged from six months to nearly two years.

The blood could not be secured by the same method throughout the whole series. Newborn animals are so small and have such a limited supply of blood that the sample was taken from the severed neck after decapitation. Thus no counts at later dates were ever made on animals counted at birth. Sometimes the blood of week-old young was secured in this manner, but more often it was from the femoral vein. Wherever possible a mouse yielded samples at the end of the first, second, third, et cetera, weeks. From two weeks on, blood was usually obtained from the severed tip of the tail, several millimeters being cut off each time a sample was secured. Occasionally the blood came from the leg if the tail would not bleed. The flexed and their normal controls were treated alike; if the sample was taken from the leg of one, it was secured from the same place on the other. The blood from the decapitated newborn mice was probably slightly diluted with lymph, but such dilution occurred both in the flexed and their normal controls, so that the method of securing the sample did not invalidate the comparison.

Mr. MIXTER used a Will certified pipette in securing samples when both erythrocytes and leucocytes were to be counted, and a Trenner pipette when the number of leucocytes only was to be determined. A 1 percent solution of sodium chloride tinged with a small quantity of Gentian Violet was used when both types of cells were counted, while a  $1\frac{1}{2}$  percent acetic acid solution tinged with Gentian Violet was utilized for the white cell

counts alone. The blood was diluted 200 times with the salt solution and only 20 times with the acid solution. The first determinations were made with a Levy-Hauser single counting chamber, the average of two successive counts being taken as the record for the animal. The Levy-Hauser double counting chamber was used in most cases, for two samples could then be obtained at one filling of the chamber. The percentage of hemoglobin was estimated by using a Tallquist color scale, which is adequate for an approximate, but not for a highly accurate determination. This scale gives the concentration (percentage) of hemoglobin in a blood sample as compared with normal human blood whose hemoglobin content is taken as 100 percent.

It should be understood clearly that Mr. MIXTER's object was to determine the cause of the pale color in most of the newborn flexed mice. It might, conceivably, have been due to an abnormally thick skin. He selected animals for the study of the blood because they were flexed tailed and not on the basis of color at birth. Consequently this is a report on the blood of flexed tailed mice and not an extensive investigation of anemia. The genetic relation of flexed tail to anemia, the histology of this type of blood, and the fetal history of anemia are problems yet to be attacked.

The erythrocytes of the flexed animals and the two types of controls will be considered first, after which the hemoglobin and leucocyte content will be discussed. Table 12 gives the average numbers of red cells per cubic millimeter of blood in the flexed and normal mice, beginning at birth, then at weekly and fortnightly intervals up to twelve weeks, and finally at the adult stage. The number of individuals used in making each computation is indicated. The data of table 12 are plotted in figure 1. Table 13 gives the frequency distribution of red blood cells at birth in the three categories of animals. The purpose of the table is to show the extent to which the flexed and their controls overlap.

Table 12 and figure 1 reveal that there was a rapid increase of erythrocytes from birth to about the fourth week in the controls and the flexed. For some reason the red cells of the homozygous normals remained nearly constant from the second to the third week, but the fourth week brought an augmentation which compensated for this lag. After about the fourth week there was a slower growth in erythrocyte content until the eighth or tenth week, when the adult level was reached. The erythrocytes of the flexed mice increased from 3,550,000 per cubic millimeter at birth to 11,360,000 at eight weeks; this latter number remained about the same at ten and twelve weeks, and at the adult stage. The heterozygous normals started at birth with a larger number of red cells (4,850,000 per cubic millimeter) than the flexed, the number increasing relatively rapidly to the fourth week, but not so rapidly as in the flexed animals, as is shown by the



TABLE 12  
*Red blood cells of fieveled and normal mice.*

AGE OF THE MICE	FLEXED MICE		HETEROZYGOUS NORMAL MICE		HOMOZYGOUS NORMAL MICE	
	AVERAGE NUMBER OF RED CELLS PER CUBIC MILLIMETER	NUMBER OF MICE USED	AVERAGE NUMBER OF RED CELLS PER CUBIC MILLIMETER	NUMBER OF MICE USED	AVERAGE NUMBER OF RED CELLS PER CUBIC MILLIMETER	NUMBER OF MICE USED
Birth	3,550,000	27	4,850,000	22	4,020,000	15
1 week	5,130,000	26	5,450,000	31	5,090,000	15
2 weeks	6,810,000	23	6,840,000	22	6,600,000	15
3 weeks	8,120,000	20	7,710,000	20	6,750,000	15
4 weeks	10,050,000	17	9,510,000	17	9,200,000	12
6 weeks	10,510,000	15	10,090,000	15	9,870,000	4
8 weeks	11,360,000	13	10,320,000	13	10,000,000	2
10 weeks	11,170,000	15	10,690,000	14		
12 weeks	11,500,000	14	10,790,000	14		
Adults	11,210,000	16	11,040,000	15	9,690,000	12

fact that the normal curve crossed the flexed at the second week. Thereafter the heterozygous normals had a lower concentration of erythrocytes than the flexed. These normals reached a level at about the tenth week which was but little lower than for the twelfth and the adult stage. The homozygous normal controls showed a birth count of 4,020,000 red cells, which was intermediate between the flexed and heterozygotes, but closer to the former than the latter. The homozygous normal and the flexed curves intersected at one week, and thereafter the homozygotes had fewer erythrocytes than either the heterozygotes or the flexed.

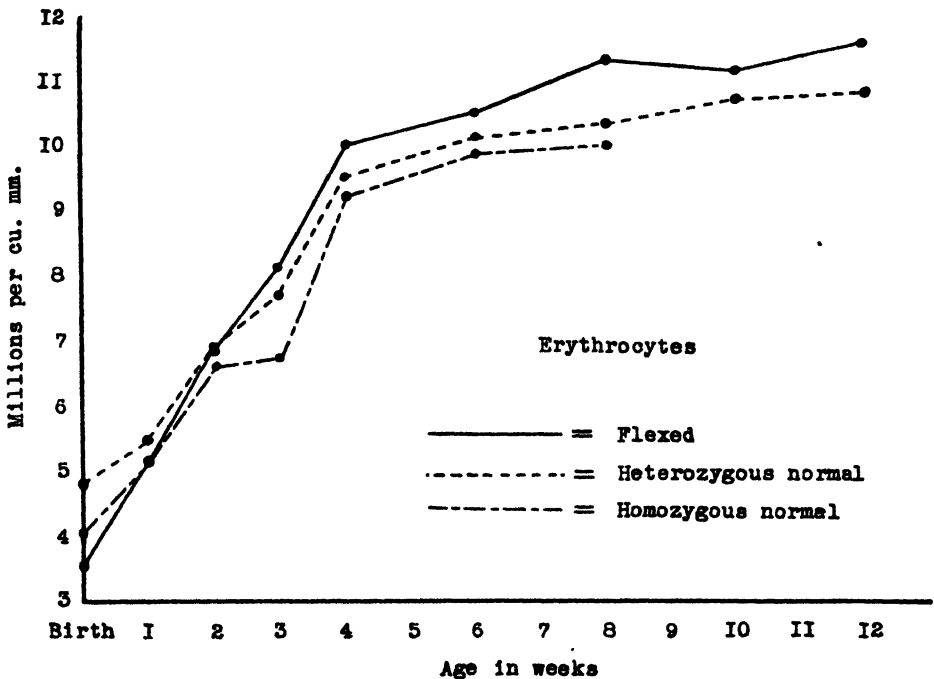


FIGURE 1.

The lack of erythrocytes which on the average afflicts the flexed animals is characteristic of early infancy, the group as a whole rapidly recovering from the disability. Let us examine critically the evidence for this statement. Table 13 shows the frequency distribution of red cell counts in the flexed and controls at birth. There was much overlapping among the three types, nearly all the homozygous normals falling within the range of variation of the flexed. The probable causes of these overlaps will be discussed later. We are not concerned for the present with the blood counts after the second week, though the apparent superiority of the flexed animals after this age may be worth further study. The average numbers of erythrocytes at birth were as follows:

Flexed: 3,550,000  $\pm$  99,900  
 Homozygous normals: 4,020,000  $\pm$  112,700  
 Heterozygous normals: 4,850,000  $\pm$  111,400

TABLE 13

*Frequency distribution of red blood cells in flexed and normal mice at birth.*

CLASSES (NUMBER OF RED CELLS PER CUBIC MILLIMETER)	FREQUENCIES		
	FLEXED	HETEROZYGOUS NORMALS	HOMOZYGOUS NORMALS
2,400,000-2,490,000	2	..	..
2,500,000-2,590,000	..	..	..
2,600,000-2,690,000	4	..	..
2,700,000-2,790,000	..	..	..
2,800,000-2,890,000	2	..	..
2,900,000-2,990,000	1	..	1
3,000,000-3,090,000	1	..	1
3,100,000-3,190,000	1	..	..
3,200,000-3,290,000	1	..	1
3,300,000-3,390,000	2	..	..
3,400,000-3,490,000	..	..	..
3,500,000-3,590,000	..	1	1
3,600,000-3,690,000	..	..	2
3,700,000-3,790,000	..	..	..
3,800,000-3,890,000	3	..	..
3,900,000-3,990,000	1	2	1
4,000,000-4,090,000	1	2	2
4,100,000-4,190,000	..	1	..
4,200,000-4,290,000	1	1	..
4,300,000-4,390,000	3	1	2
4,400,000-4,490,000	..	..	..
4,500,000-4,590,000	1	1	1
4,600,000-4,690,000	1	2	..
4,700,000-4,790,000	1	..	..
4,800,000-4,890,000	1	..	..
4,900,000-4,990,000	..	..	3
5,000,000-5,090,000	..	2	..
5,100,000-5,190,000	..	2	..
5,200,000-5,290,000	..	1	..
5,300,000-5,390,000	..	1	..
5,400,000-5,490,000	..	1	..
5,500,000-5,590,000	..	..	..
5,600,000-5,690,000	..	..	..
5,700,000-5,790,000	..	1	..
5,800,000-5,890,000	..	..	..
5,900,000-5,990,000	..	1	..
6,000,000-6,090,000	..	1	..
6,100,000-6,190,000	..	..	..
6,200,000-6,290,000	..	..	..
6,300,000-6,390,000	..	..	..
6,400,000-6,490,000	..	..	..
6,500,000-6,590,000	..	1	..

The difference between the flexed and the homozygous normals was  $470,000 \pm 151,000$  erythrocytes per cubic millimeter. This difference was only 3.1 times as large as its probable error, so it was just barely significant statistically according to conventional standards. The difference between the flexed and the heterozygotes was  $1,300,000 \pm 150,000$ , and since this difference was 8.7 times its probable error it can scarcely have been due to chance. Thus the flexed animals are definitely deficient in erythrocytes at birth.

The evaluation of these differences should take into account the nature of the controls. The heterozygous normals were, theoretically, better controls than the homozygotes. This was particularly true of the newborn young. Usually the blood count of a flexed animal was paired with the count for a normal litter mate. Thus a flexed animal at birth had been subjected, as a rule, to about the same prenatal environmental influences as the normal heterozygous mouse which was selected to serve as its control, for both had developed in the same uterus at the same time. The homozygous controls, of course, were always developed in uteri which were carrying no flexed mice. Their uterine environment may have been quite different, on the whole, from that of the flexed animals with which they were compared within a few hours after parturition. Also, the heterozygous normal controls were probably, on the whole, genetically more like the flexed animals than were the homozygotes, for the heterozygotes came from the same group of parents as the flexed. So if there are factors other than the one with which we are now concerned that affect the number of erythrocytes and the amount of hemoglobin, the heterozygotes would be expected to resemble the flexed in the possession of these factors more closely than the homozygotes.

Thus it is conceivable, though of course not proved, that some of the homozygous normal controls may have possessed factors, other than the main factor for anemia, which depressed the erythrocyte content at birth, and that such factors were less frequent in the flexed animals and the closely related heterozygous controls. This might account for the relatively small difference between the flexed and homozygotes at birth. Such an explanation of the higher counts in the heterozygous normals at birth in contrast with the homozygotes and the flexed is, I think, more plausible than an assumption to the effect that heterozygosity with respect to the flexed gene raises the erythrocyte count over the normal level in some mysterious manner.

To conclude, flexed animals as a group are lacking in red blood cells at birth, but on the average they reach the normal level when one to two weeks old. This anemia is not a permanent or fatal defect.

The facts with regard to hemoglobin are presented in tables 14, 15, and

TABLE 14  
*Hemoglobin of flexed and normal mice.*

AGE OF MICE	FLEXED MICE		HETEROZYGOUS NORMAL MICE		HOMOZYGOUS NORMAL MICE	
	AVERAGE PERCENTAGE OF HEMOGLOBIN	NUMBER OF MICE USED	AVERAGE PERCENTAGE OF HEMOGLOBIN	NUMBER OF MICE USED	AVERAGE PERCENTAGE OF HEMOGLOBIN	NUMBER OF MICE USED
Birth	38.2	44	60.3	41	56.9	26
1 week	49.0	43	57.9	46	53.3	15
2 weeks	54.0	36	56.3	31	56.0	15
3 weeks	58.2	36	57.0	33	52.0	15
4 weeks	58.2	31	61.5	30	60.8	12
6 weeks	60.7	15	61.7	15	61.3	4
8 weeks	65.4	13	66.5	13	60.0	2
10 weeks	65.0	15	64.3	14	....	..
12 weeks	67.1	14	67.5	14	....	..
Adults	68.8	16	64.3	15	65.8	12

TABLE 15  
*Frequency distribution of hemoglobin in flexed and normal mice at birth.*

CLASSES (PERCENTAGE HEMOGLOBIN)	FREQUENCIES		
	FLEXED	HETEROZYGOUS NORMALS	HOMOZYGOUS NORMALS
30	13	..	..
35	7	..	..
40	10	2	..
45	12	..	2
50	1	1	2
55	1	3	8
60	..	25	13
65	..	6	..
70	..	3	1
75	..	..	..
80	..	1	..

TABLE 16  
*Frequency distribution of hemoglobin in flexed and normal mice at one week of age.*

CLASSES (PERCENTAGE HEMOGLOBIN)	FREQUENCIES		
	FLEXED	HETEROZYGOUS NORMALS	HOMOZYGOUS NORMALS
35	1	..	..
40	5	..	..
45	14	..	..
50	11	9	7
55	6	11	6
60	6	19	2
65	..	6	..
70	..	..	..
75	..	..	..
80	..	1	..

16, and in the curves of figure 2. As previously mentioned, the Tallquist method was used in determining the relative concentrations of hemoglobin. Table 15 shows the frequency distribution for the flexed and normal mice at birth, while table 16 gives such data at the age of one week. Hemoglobin is the substance which gives the blood its characteristic red color, so if the paleness of the infant mice is due to an anemia, one would expect to find less hemoglobin in the blood of the newborn flexed than in the two groups of controls. Such was the case.

The flexed animals had at birth an average hemoglobin concentration of 38.2 percent of the normal human standard. This increased rapidly during the first three weeks, reaching the level of the controls during the third

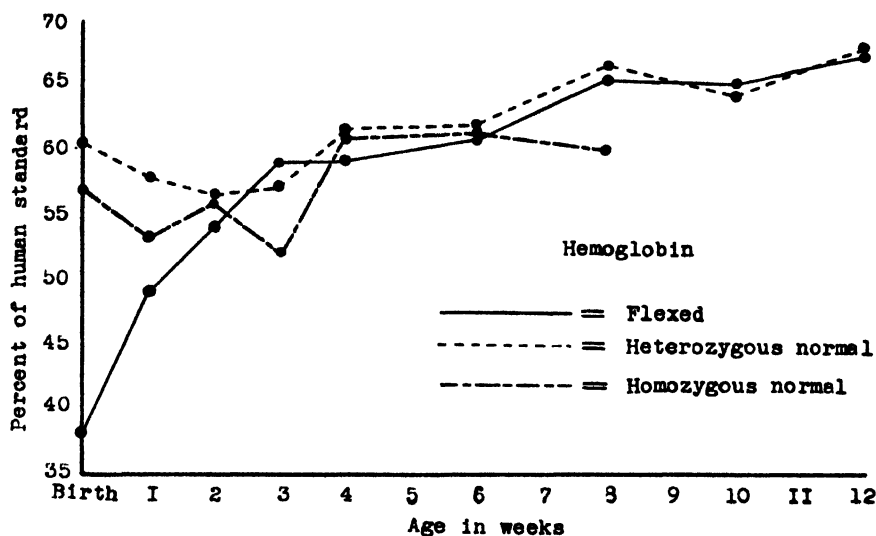


FIGURE 2.

week, thereafter slowly increasing until an adult percentage of 68.8 percent was attained. The heterozygous normal mice began postnatal life with an average hemoglobin percentage of 60.3. This decreased slightly during the first and second weeks, then came a gradual but irregular rise which carried the hemoglobin percentage to 64.3 percent in the adult. The curve for these heterozygous controls followed closely the curve for the flexed from the third to the twelfth week, though the flexed adults had 4.5 percent more hemoglobin than the adult controls. The flexed animals increased their hemoglobin by over 30 percent from birth to adulthood, while the corresponding increase for the heterozygous normals was only 4 percent. The homozygous normals had 56.9 percent of hemoglobin at birth; the percentage declined irregularly until the third week, after which came a rise which carried the hemoglobin content to 65.8 percent in the adult. No observations were made at the end of the tenth and twelfth weeks. The

more irregular course of the curve for the homozygous controls may have been due to the relatively small numbers of individuals observed. The hemoglobin content of the homozygotes at birth was, as with the erythrocyte counts, intermediate between the flexed and the heterozygous controls. It is worthy of note, also, that though the flexed mice enter the world markedly deficient in hemoglobin, on the average, yet the survivors not only speedily overtake the normals when about two weeks old, but on the whole slightly exceed them at maturity. Thus the anemia from which the flexed mutants suffer automatically disappears during infancy, in this respect differing decidedly from the severe lethal anemia found by DE ABERLE in homozygous dominant white animals.

Table 15 shows that there was some overlapping in the distributions of flexed and control groups at birth, but this was not as pronounced as with the distributions of erythrocyte counts presented in table 13, which shows that the homozygous normals fell almost completely within the range of variation of the flexed, and that half the heterozygous normals did the same. In table 15 only 15 percent of the heterozygous and 46 percent of the homozygous normals come within the limits of the flexed distribution. Moreover, the differences between the flexed animals and the controls at birth were statistically significant, as the following computations show.

Average percentage of hemoglobin for the flexed:  $38.2 \pm .68$  percent

Average percentage of hemoglobin for the homo-

zygous normal controls:  $56.9 \pm .69$  percent

Average percentage of hemoglobin for the hetero-

zygous normal controls:  $60.3 \pm .70$  percent

The difference between the flexed and homozygous normal controls was  $18.7 \pm .97$  percent, and since this difference was 19.3 times as large as its probable error it was highly significant statistically. The percentage for the heterozygous straights exceeded that for the flexed by  $22.1 \pm .98$  percent. This difference was likewise of great significance because it was 22.6 times the size of its probable error. Thus the hemoglobin percentages emphatically testify that, on the average, the flexed group was anemic when born.

The flexed and anemic animals were more nearly alike at one week of age than at birth, but the differences were still significant, as the following data show.

Average percentage of hemoglobin for the flexed:  $49.0 \pm .66$  percent

Average percentage of hemoglobin for the homo-

zygous normal controls:  $53.3 \pm .61$  percent

Average percentage of hemoglobin for the hetero-

zygous normal controls:  $57.9 \pm .57$  percent

The percentage of hemoglobin in the heterozygous controls was  $8.9 \pm .87$  percent higher than in the flexed animals. This difference was over ten

times the magnitude of its probable error. The homozygous normals had  $4.3 \pm .90$  percent more hemoglobin than the flexed, and this difference too was significant because it was 4.8 times as large as its probable error.

Thus the flexed animals attain the hemoglobin level of the normals at about the second week, while the number of erythrocytes reaches the normal level perhaps a little earlier (fig. 1), but all the data show that the flexed animals are on the average deficient in erythrocytes and hemoglobin at birth, but that these defects are rapidly remedied.

An examination of figures 1 and 2 shows at a glance that the individual erythrocytes of both types of controls were presumably, on the average, more richly endowed with hemoglobin at the time of the mouse's birth than were the erythrocytes of the flexed individuals. The red cell curves of all three groups follow much the same course, a rapid infantile increase being followed by a lower rate. This was not the case with the hemoglobin percentages. The hemoglobin and erythrocytic curves of the flexed animals resemble one another, but the hemoglobin percentages of the two groups of controls did not show the marked infantile increase which was found in the flexed group. Whereas the flexed animals were 30.6 percent below their adult level, the controls had only 4.0 percent and 8.9 percent less hemoglobin at birth than when full grown.

Quantitative estimates of the relative average amounts of hemoglobin in each erythrocyte of the control types and flexed at birth, and of the adults will be of interest. However, the reader is cautioned not to take these estimates too seriously, for the numbers of mice used were not large; also, as will be pointed out shortly, the controls may possibly have included a few anemic animals and the flexed group a number of normal blooded individuals.

Suppose we define a unit of hemoglobin as the average amount carried by an erythrocyte of a flexed animal at birth. Suppose, further, that we use throughout the computations the volume of blood which in a newborn flexed mouse contains 100 erythrocytes. We may now find, by using the data in tables 12 and 14, how many units of hemoglobin, on the average, an erythrocyte of a flexed animal carries when adult. The hemoglobin of flexed mice increased from birth to adulthood by 80.1 percent of the amount present at birth. Thus there would be 180.1 units of hemoglobin ( $100 + 80.1$ ) in the volume of adult blood used as our standard volume. The number of erythrocytes, during this period, increased by 215.8 percent of the number present at birth, so that there would be 315.8 erythrocytes per unit volume of blood in the adult ( $100 + 215.8$ ). The average hemoglobin quota of the adult's erythrocytes in flexed mice would therefore be,  $180.1 \text{ units} \div 315.8 \text{ erythrocytes} = .57 \text{ units}$ . Thus the hemoglobin load of each erythrocyte decreased from 1.00 unit at birth to .57.



The hemoglobin per red cell at birth in the heterozygous normal controls is computed as follows. These controls had 1.579 as much hemoglobin per unit volume of blood at birth as did the flexed (60.3 percent, hemoglobin at birth of the controls  $\div$  38.2 percent, hemoglobin at birth of the flexed). The heterozygous controls had also 1.366 as many erythrocytes per unit volume of blood at birth as the flexed mice (4,850,000, erythrocytes at birth of the controls  $\div$  3,550,000, erythrocytes at birth of the flexed). The number of units of hemoglobin per erythrocyte for the heterozygous normals when born would be, therefore,  $1.579 \times 100 \div 1.366 \times 100 = 1.16$  units.

To determine, next, the units of hemoglobin per red blood cell of the adult heterozygous controls, we make use of the facts that the hemoglobin of these increased over the content at birth by 6.6 percent of the amount at birth, and that the increment for the erythrocytes was 127.6 percent of the number at parturition. Thus the average hemoglobin quota in the red blood cell of the adults was  $157.9 + (157.9 \times .066) \div 136.6 + (136.6 \times 1.276) = .54$  units.

The following summary brings together all the estimates from the available data.

Units of hemoglobin per erythrocyte for

	Flexed	Heterozygous normal controls	Homozygous normal controls
At birth	1.00	1.16	1.32
In adults	.57	.54	.63

The adult erythrocytes in all three types of animals are thus seen to have carried about the same quantities of hemoglobin, the flexed being intermediate between the controls. On the other hand, the controls' red blood cells were endowed at birth with from 16 percent to 32 percent more hemoglobin per cell than the flexed animals. Thus the blood of the newborn mutants was not only deficient in total hemoglobin content, and in erythrocytes, but each erythrocyte carried less than the normal quantity of hemoglobin. These estimates are probably of slight quantitative value, yet they seem to bring out another important fact about our flexed mutation.

Mr. MIXTER studied the leucocyte content also of his animals. His findings are summarized in table 17, which gives the average numbers of leucocytes per cubic millimeter at the different ages indicated from birth to maturity. All three types showed an irregular increase with advancing age, but the flexed animals did not differ significantly from the controls. The blood deficiencies of the flexed did not therefore involve the white cell content as a whole. Mr. MIXTER did not enumerate separately the different types of leucocytes, however, so it is conceivable that a deficiency of one type of leucocyte in the flexed may have been offset by an increase in another type.

TABLE 17  
*Average numbers of leucocytes per cubic millimeter.*

AGE	FLEXED		HETEROZYGOUS NORMAL		HOMOZYGOUS NORMAL	
	NUMBER OF CELLS	NUMBER OF INDIVIDUALS	NUMBER OF CELLS	NUMBER OF INDIVIDUALS	NUMBER OF CELLS	NUMBER OF INDIVIDUALS
Birth	4297 $\pm$ 202	16	4129 $\pm$ 238	16	3993 $\pm$ 229	26
1 week	4192 $\pm$ 139	15	4481 $\pm$ 201	15	5000 $\pm$ 326	14
2 weeks	5086 $\pm$ 354	15	6167 $\pm$ 586	11	5000 $\pm$ 360	15
3 weeks	5805 $\pm$ 385	15	6502 $\pm$ 323	15	5600 $\pm$ 547	17
4 weeks	7435 $\pm$ 434	12	5340 $\pm$ 477	12	5600 $\pm$ 657	12
6 weeks	7700 $\pm$ 486	8	8000 $\pm$ 759	8	4500 $\pm$ 370	4
8 weeks	11200 $\pm$ 1255	9	8700 $\pm$ 595	10	.. ..	..
10 weeks	8700 $\pm$ 690	12	10600 $\pm$ 1106	11	.. ..	..
12 weeks	12700 $\pm$ 846	12	11800 $\pm$ 668	12	.. ..	..
Adult	10000 $\pm$ 834	15	9600 $\pm$ 748	13	11000 $\pm$ 738	12

#### ARE THE FLEXED AND ANEMIC CHARACTERS DUE TO THE SAME GENE?

We may now inquire whether the characters flexure and anemia are the results of a single recessive gene or of two recessive genes which are closely linked. This question can not be answered from the data at hand, but there are certain suggestive facts. If the flexed gene causes the anemia, then anemia and flexed should always occur together (unless some agencies, genetic or environmental, can suppress one of these characters without eliminating the other), and a normal tail would always be associated with the absence of this type of anemia. In other words there would be no cross-overs. But there is enough evidence at hand to shake one's faith in such a theory. Individuals that may possibly be the results of crossing over between genes for anemia and flexed were found.

Tables 2 and 4 contain such cases (see HUNT, MIXTER, and PERMAR, 1933). Among the 963 newborn backcross progeny reported in table 2, 78 are recorded as anemic but normal tailed. There are 1478 newborn  $F_2$  mice in table 4, and of these 81 were normal tailed and anemic. Such a large number of exceptional cases would be good evidence that crossing over between genes for flexed and anemia had occurred if the classification of the newborn young could be depended on, but it can not. The identification of flexures in the tails of very young mice is sometimes difficult or impossible. Flexed is a highly variable character, as has been shown already. It grades into normality so perfectly that no absolutely defined boundary can be designated. Mr. MIXTER reports that among 415 offspring from flexed  $\times$  flexed crosses, 3 anemic young were observed which at the age of 21 days showed no caudal stiffness or flexure. If such conditions are encountered in weaned flexed mice, it is obvious that the

absence of a bend in the very pliable tail of a newborn mouse does not prove it to be normal tailed.

The uncertainty in classifying the tails at birth is brought out by a study of the following 100  $F_2$  young which were branded with a hot needle on the day of birth, then observed when they reached the age at which the animals were usually given their final rating.

Normally red at birth, normal tailed at birth, and normal tailed at the final count:	75
Normally red at birth, doubtfully normal tailed at birth, and normal tailed at the final count:	12
Normally red at birth, straight tailed at birth, and doubtfully normal tailed at the final count:	1
Probably anemic, straight tailed at birth, and normal tailed at the final count:	1
Doubtfully anemic, straight tailed at birth, and doubtfully flexed at the final count:	1
Anemic, flexed at birth, and flexed tailed at the final count:	5
Anemic, doubtfully straight tailed at birth, and flexed tailed at the final count:	2
Anemic, doubtfully flexed at birth, and flexed tailed at the final count:	2
Anemic, straight tailed at birth, and the tail form transitional at the final count:	1

It will be seen that in five cases the tail was described as straight or doubtfully straight at birth, and that this verdict was reversed or rendered doubtful when the final count was made. Thus at least some of my newborn mice which were described as straight tailed anemics at birth may actually have been flexed, so the evidence from such cases does not prove that crossovers have occurred between genes for flexure and anemia.

Tables 2 and 4 record 5 flexed tailed newborn individuals (3 of them backcross young and 2  $F_2$ 's) which appeared to show the normal redness of non-anemic animals. Two of these turned out to be normal tailed, for no deaths occurred in the litters to which they belonged, and at the final counts after weaning, all the animals in their litters were found to have normal tails. This left three cases of supposedly flexed young which seemed normal blooded when the newborn were examined. In view of the fact that an ordinary muscular bend in the tail of an infant mouse might occasionally be taken for a flexure, no conclusions can be drawn from these three cases.

The writer found two newborn mice in a litter, not elsewhere recorded in this paper, both of which had flexed tails and a normal red color. Mr. MIXTER's examination of the bloods showed one to have 5,890,000 red cells per cubic millimeter with 55 percent hemoglobin, and the other 65 percent hemoglobin and 5,300,000 red cells. Reference to tables 13 and 15 shows that the first animal had a normal erythrocyte count and a hemoglobin percentage which was at the extreme upper limit of the flexed distribution, while the second one was normal as regards hemoglobin percentage and red cell content, being entirely outside the range of flexed animals in both respects. To sum up the matter, the data are too scant and in many respects too uncertain to demonstrate whether or not the flexed and anemic traits are due to separate genes.

If recovery from anemia begins before birth, an occasional newborn flexed animal might have normal blood.

It should be kept in mind that Mr. MIXTER studied the blood of flexed animals rather than of anemics, so that his quantitative findings are not accurately descriptive of anemia. When newborn young were selected for blood counts and hemoglobin determinations, they were classified as flexed or straight tailed. Reference to tables 2 and 4 will show that there were quite a number of "intermediate or doubtful" cases where the writer was unable to classify the tails in newborn young. Considerable experience is required for this work. The tail may be bent as a consequence of muscular contraction on one side, or it may have a slight flexure which can be identified only after a rather close scrutiny. One of the main characteristics which the writer has assumed to identify newborn flexed tailed animals is a rather distinct angularity in the region of the flexion, usually quite different in appearance from the curvilinear aspect of a normal tail whose muscles are bending it. But there are undoubtedly cases where even experienced observers would not agree on the classification at birth. That is why it is so important to defer, as we have, final judgment as to the number of flexed animals in a litter until it is three to four weeks old, when the flexed condition can usually be identified positively.

Thus it is conceivable that Mr. MIXTER may have classified as flexed some newborn mice that were actually normal tailed. If the same gene causes both the flexed and anemic characters, such classification would place some normal blooded animals in the flexed category, thus causing the distributions of erythrocytes and hemoglobin percentages for flexed animals to overlap the ranges of both controls. Tables 13 and 15 show that such overlappings occur. Likewise, he may have classified as normal some animals that were homozygous flexed. This could happen wherever the genetically flexed animal would have shown only stiffness when full grown. Such events would extend the distributions of blood counts and hemoglo-

bin percentages of the heterozygous normal controls over into the ranges of the flexed animals. The above theory might account for the overlapping of the flexed and normal distributions of hemoglobin percentages, but it is difficult to believe that it explains the overlaps in the erythrocyte counts for flexed and homozygous normals, because their distributions cover almost the same range. Further study of the blood of newborn mice is needed to establish accurately the relationship between blood content and the paleness or redness of the mouse at birth.

#### MORTALITY OF FLEXED ANIMALS

One of the by products of Mr. MIXTER's study of inbreeding was considerable information about deaths among infants in the second generation of inbred flexed animals. Unfortunately, normal controls bred and reared at the same time and under strictly comparable environmental conditions were not available, so that positive conclusions can not be drawn. The facts will be given, however. The record of deaths is as follows:

TOTAL NUMBER OF 2ND GENERATION MICE AT BIRTH (ONE ESCAPED)	NUMBERS DYING AT					TOTAL DYING	PERCENTAGE DYING
	1-3 DAYS	4-7 DAYS	8-14 DAYS	15-21 DAYS	22-28 DAYS		
625	79	60	32	14	17	202	32.3

About one-third of these flexed infant mice died before they were 28 days old. This seems like an exceedingly high mortality, presumably higher than the normal rate. The deaths were most numerous during the first three days, then sharply declined. It is worth noting that 84.7 percent of these deaths occurred during the first two weeks, the period of anemia, suggesting that this may have been an important contributory cause. Whatever the reasons are for this high mortality, it appears probable that young flexed mice are lacking in vitality, and this view is consistent with the fact that the writer has had some difficulty in maintaining the flexed stock.

The flexed mutation presents some interesting similarities to, and contrasts with, the hereditary anemia described by DE ABERLE (1927) in homozygous dominant white mice. DE ABERLE's animals weighed less than normals, as did ours also (Mr. ALEXANDER A. ANDREWS' unpublished data on flexed), and both were much lighter in color than newborn normal mice. The anemia in the homozygous dominant whites was very severe, for all the afflicted young died within ten days after birth. Our flexed animals probably have a higher death rate than normals during infancy, but they recover from the anemia while still young. We can not accurately compare the blood of DE ABERLE's and our anemics because, as has been

said already, the blood of flexed, rather than of anemic, mice was studied by Mr. MIXTER. DE ABERLE's newborn normal mice had an average of  $4,740,020 \pm 117,932$  red cells per cubic millimeter, while the newborn anemics' average was  $663,009 \pm 15,714$ . Her normal controls had  $89.78 \pm 1.59$  percent of hemoglobin, as judged by human standards, while the percentage for the anemics was  $22.13 \pm .56$  percent.

#### SUMMARY

1. The anemia in the flexed tailed strain of mice is probably a recessive character.

2. Flexed animals are on the average deficient at birth both in hemoglobin and erythrocytes, but this defect disappears, on the whole, at about the age of two weeks.

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# CROSSING OVER AND GENE REARRANGEMENT IN FLOWERING PLANTS<sup>1</sup>

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## HYPOTHESES OF CROSSING OVER

### *Kinds of gene rearrangement*

"Crossing over" exists apparently in all those flowering plants which have been sufficiently investigated with regard to it. (By itself, it does not rearrange different genes, but only exchanges alleles.) Genetically it consists in exchange of normally equal and homologous segments of a gene string (MORGAN 1911, STURTEVANT 1915, MULLER 1916). It has been proved, by the microscope, to consist in the exchange, at meiosis, of normally equal portions of homologous chromonemas (CREIGHTON and McCLINTOCK 1931, STERN 1931). It may thus be termed "homologous exchange". Since the process is a normal one, the mechanism is apparently so perfect that no genes are lost in the exchange.

"Reversed crossing over" consists in the exchange of different arms of homologous chromosomes, apparently at the fusal (spindle) constriction. It has been proved to occur at the origin of the secondary mutants of *Datura*, usually in trisomics, and probably at meiosis.

"Reciprocal translocation" (heterologous interchange) is much rarer than crossing over. It consists in the mutual substitution of usually unequal segments of heterologous chromosomes. It has been proved to occur cytologically as well as genetically (see BELLING 1927, STURTEVANT and DOBZHANSKY 1930, DOBZHANSKY and STURTEVANT 1931, McCLINTOCK 1930). It seems to originate at both mitosis and meiosis. It may be caused by X-rays. Apparently genes may sometimes be lost in the process.

"Translocation" shows a segment of one chromosome terminally attached, usually to a heterologous chromosome (see especially PAINTER and MULLER 1929).

"Inversion" consists in a portion of the gene string or chromonema being turned round, end for end; genes being apparently sometimes lost in the process (see especially STURTEVANT 1931, and McCLINTOCK 1931).

"Deletion" removes a non-terminal portion of the gene string, the gap closing up (see especially PAINTER and MULLER 1929). The portion removed may form a ring (McCLINTOCK 1932).

<sup>1</sup> This paper gives the results of cytological work done under the auspices of the CARNEGIE INSTITUTION OF WASHINGTON. It is published posthumously and out of the order of receipt at the expense of the institution.

"Insertion" causes a segment of one chromonema to be placed within another gene string. Genes may be lost in the process. It seems quite rare.

"Deficiency" is usually the loss of a (terminal) segment of the gene string (see McCLINTOCK 1931). It originates at meiosis or mitosis.

### *The hypotheses*

A successful hypothesis is normally based on *verae causae* (ascertained facts), while unsuccessful hypotheses are often marked by containing one or more *factae causae* (imagined statements). The first hypothesis as to crossing over seems to have been that of JANSSENS (1909), who predicted exchange of parts of homologous chromosomes, from his observations of chiasmata. In this hypothesis he introduced the *factae causae* of two strands first breaking accurately at corresponding points, and then these same two strands joining in an alternate way. These processes took place conjecturally at an overlap (or at a half twist). This "break and join" seems to the writer improbable mechanically. There would be needed an accuracy of position, in the two strands, of less than half a micron, with nothing to mark the breakage points. Without such accuracy, the crossing over would be unequal; which it is not (with one exception). Also the conditions required for a break are the reverse of those for a join.

A second hypothesis, stated in full by BATESON in 1911, demanded selective division of cells (somatic and gonial), in accurate ratios, precisely separating the pairs of alleles, and properly multiplying them, so as to result in numbers corresponding to the mathematical expression of certain combinations. This would seem to require unknown forces to bring it about, and was also contrary to the known relevant facts. It has been proved to be wrong.

A third composite hypothesis for crossing over required openings-out at diplotene, alternately at the primary split and the secondary split; these alternations being the *cause* of chiasmata. (It is possible that such alternations may occur as a *consequence* of the opening out of chiasmata at diplotene, in plants like *Datura*.) It also adopted the "break and join" of JANSSENS, the weak point of his hypothesis (SAX 1932). This alternate-opening hypothesis is negatived for certain liliaceous plants (and for *Dendrocoelum*) by the observed facts: that chiasmata are present at pachytene; that it is recognizably the primary split which opens out at early diplotene (a stage which has rarely been well figured); while the secondary split at this stage is responsible only for a two-lobed state of the chromomeres (GELEI 1921, BELLING 1931a). The sequence of these splits is especially clear in *Lilium* and *Fritillaria*, where the primary split can be followed from early pachytene to mid diplotene. This third hypothesis



thus contains three imaginary causes: alternate openings-out as a cause, not a consequence, of chiasmata; accurate double breaks; and special double joinings. Chromonemas have not been observed to break and join again at the chiasmata, as required by this hypothesis.

A fourth hypothesis, that of the present writer, attempts to postulate *verae causae*. It was framed after a study of the chromomeres and chromioles; and this was not the case with the three other hypotheses, which ignored the chromomeres. It also accounts for gene rearrangements, which the other hypotheses ignore. The chief supports of this fourth hypothesis are the following. (a) The absence of longitudinal division in the chromonema at leptotene; as observed especially by the writer (1931a), WENRICH (1917), GELEI (1921), and BELAR (1929a). (b) The proof of the complete equivalence of the two chromioles resulting from the secondary split in each chromomere (MENDEL 1866, SUTTON 1903, GELEI 1921). (c) The fact that only homologous chromomeres, including alleles, are connected transversely (that is, synapse) at zygotene; as observed by GELEI (1921), by the writer (1928, 1931a), and also by MCCLINTOCK (1931) in cases of inversion. (d) The fact that non-homologous chromomeres are connected longitudinally to their nearest neighbors by one, and only one, fiber; as observed especially by GELEI (1921) and by the writer (1928, 1931a). (e) That the *two* chromioles formed from each original chromomere by the secondary split are seen to have acquired a new fiber connection longitudinally (in addition to the old fiber), which joins *one* of them to the nearest non-homologous chromiole each way (GELEI 1921, BELLING 1928, 1931a, 1931b). (f) That such new connections are formed when the chromonema is dividing longitudinally (BELLING 1931a, 1931b). (g) That crossing over (exchange), inversion, translocation, interchange, and deletion can take place when the chromonemas are dividing longitudinally (BELLING 1931b).

#### PRELIMINARY KARYOLOGIC EVIDENCE

*Chromonemas are normally unsplit at leptotene and during the resting stage*

The writer has made a special investigation of this point, by means of smear preparations in iron-aceto-carmin, and also by smear preparations fixed with chromic-acetic-formalin and stained with iron-brazillin. The plants mostly used were *Tradescantia virginiana*, *Rhoeo discolor*, *Allium triquetrum*, *Lilium regale*, *Scilla sibirica*, and *Aloe striata*. The work resulted in the conclusion that the so-called telophasic split (SHARP 1929, ROBERTSON 1931, and KAUFMANN 1925) did not exist here; and that the spongio-reticular structures in the nucleus, commonly figured in sections from mass fixations, were artifacts of slow fixation. BELAR (1929a, 1929b)

came to a similar conclusion. The writer found (like MARTENS 1927, 1929, and BELAR 1929a) that living resting (non-dividing) nuclei (in cells showing cyclosis), which would not normally divide again, when examined in an aqueous medium by the best water-immersion objectives, with accurate microscopy, were seen to contain unsplit, loosely coiled and zigzagged chromonemas. The plant material in which this was observed included: stamen-hairs of *Tradescantia virginiana*, of *Tradescantia fluminensis* (in which the nuclei sometimes appeared blank, but at other times showed the chromonemas), and of *Rhoeo discolor*; the hairs on the labellum of *Cypripedium pubescens*, and the stinging hairs of *Urtica gracilis*. When such non-dividing nuclei were fixed and stained, with optimum fixation, the same unsplit chromonemas were seen in them; but with slow fixation the familiar spongio-reticular structure (seen in sections of material fixed in mass) regularly appeared.

Preparations made by sectioning from material fixed in bulk suffer from too slow fixation. In the telophases, chromatin is being lost, and it is not strange that the interior of the vanishing chromosome should sometimes be more or less unstained, and that by poor fixation a moniliform aspect should be caused. It seems to the writer premature to infer from such changes that there is a longitudinal split in the telophase. For no signs of such a split are seen in good smear preparations of telophases, as observed by BELAR (1929b) and by the writer.

In the leptotene of *Lilium*, *Galtonia*, *Allium*, *Scilla*, *Hyacinthus*, *Tulipa*, and *Agapanthus*, in smear preparations stained with iron-brazilin and showing the chromomeres, it was ascertained by the writer that there was no trace of a longitudinal or other split, in either the chromomeres or their connecting fibers. It was also seen clearly that the secondary split came subsequently, in mid pachytene; early pachytene having only the primary split (figure 1). In *Allium*, slow destaining (in hyrax) of the early pachytene (1931a) proved that each chromomere had only one stained submicroscopic core, which was, in the writer's opinion, a gene with a thin covering of chromatin. There being only one such core in a chromomere, in the early pachytene, proved, apparently conclusively, that the secondary split had not yet appeared.

Hence, the evidence from the best fixed preparations, namely smear preparations showing chromomeres, is against any general telophasic split; and the supposed split can be sometimes explained as an artifact of slow fixation, resulting from fixation in mass.

*Crossing over probably occurs during pachytene*

In *Lilium* the average longitudinal distance between the centers of adjacent chromomeres at late pachytene (figure 2) was under half a micron. It

was seen to be less than this in several other liliaceous plants (but not in *Fritillaria*). Hence a relative movement of the homologous chromonemas by half a micron would bring about unequal crossing over, if any crossing over should occur. But unequal crossing over seems rare, being only known in the bar locus. Thus, apparently, crossing over must take place at pachy-



FIGURE 1.—Early pachytene of *Lilium regale*. Smear preparation, fixed in Navashin's mixture, stained with brazilin, and mounted in immersion oil. Pressed nearly flat. Photographed with Zeiss apochromatic 90 of 1.4 aperture, with homal. Enlargement near 1300. The chromomeres have *nearly* all synapsed. The secondary split has not yet appeared.



FIGURE 2.—Late pachytene of *Lilium regale*. Preparation as in figure 1, but not pressed. Focus somewhat below upper surface. All chromomeres have previously synapsed, and all are bilobed by the secondary split.

tene, when the homologous chromonemas are *fixed* together in the right positions by the connections between homologous chromomeres across the primary split. Any opening-out of the primary and secondary splits, or any sliding of the chiasmata, would apparently prevent that exact correspondence of the homologous chromomeres in the two homologous chromonemas, necessary to obviate unequal crossing over.

*The two chromioles formed by the secondary split are equivalent*

When a chromomere with its contained gene divides into two, genetic work has shown that there is no difference between the two products. One is apparently a replica of the other, as far as the evidence goes. Hence neither of them can truly be called the "old" chromomere or chromiole, or the "new" chromomere or chromiole. The same applies to chromonemas, which are strings of chromomeres. Two adjacent chromomeres at a certain minute distance apart, which is usually less than half a micron from center to center, can mutually form a connecting fiber. Such a fiber appears at zygotene between approaching homologous chromomeres, at first (sometimes) in the form of minute projections from the two adjacent chromomeres. Only one such fiber unites a pair of homologous chromomeres or a pair of chromioles. When the chromomeres divide at the secondary split, at mid pachytene, then a new fiber passes between two of the homologous chromioles transversely to the primary split. The new and the old fiber may thus form two transverse fibers at each locus. When the secondary split appears, a new *longitudinal* fiber is also formed. This passes between two adjacent non-homologous chromioles. The old fiber connects the other two adjacent chromioles. In all cases these fibers pass *the shortest way*, between chromioles that are nearly or quite touching; and do not pass diagonally, because this would be about four-tenths longer. (Thus there would not be normally half twists formed between sister chromonemas.)

*Opening-out at earliest diplotene is (sometimes) at the primary split, and at first at many points*

At earliest diplotene (schizotene) in *Allium triquetrum*, the homologous chromonemas of the nine bivalents begin to separate at about two hundred points (1931a), a number which is in excess of the total chiasmata, which may amount to near twenty at late diaphase. A large number of points of opening-out has also been seen at early diplotene (schizotene) in *Lilium*. At this stage, some of the pachytene chromonemas will not yet have opened-out completely, and all details of the process of opening-out can be traced (figure 3). In no case has the secondary split been seen to open-out in *Lilium*. (The chiasmata become visible at pachytene, before any opening-out.) The primary split is broad and clear, while the secondary split is hard to see (and has been so from its origin at mid pachytene), only consisting in the two-lobed condition of the chromomeres. In no case in liliaceous plants did four separate "opened-out" threads appear, as it would seem (as already stated) might occur at the points between the alternate openings-out presumed to exist in certain grasshoppers. McClinrock (1931) proved that, in deficiencies in maize, only the primary split

showed at late pachytene at the point where the deficiency was. The same was the case with inversions. If the secondary split also opens out at diplotene, it is in bivalents which, at diaphase and metaphase, show normally no chiasmata but only terminal junctions (*Datura*).

*In the supposed "break and join" of two or more chromonemas, the "break" apparently does not occur*

In crossing over, translocation, interchange, inversion, and deletion, it has been supposed that the chromonemas first broke and then joined up in a different way. Since the breaks must correspond, in crossing over (as already stated), with an accuracy of less than half a micron, they are unlikely. Since breaks are presumably due to a tension, such tension will



FIGURE 3.—Stage (*L. pardalinum*) between pachytene and diplotene (early diplotene or "schizotene"). Prepared as in figure 1, but photographed with objective of 1.3 aperture. Slightly flattened. Focus just below surface. Thick (double) and thin (single) threads. In *Lilium* the opening-out seems to be only at the primary split.

render immediately subsequent joins improbable. Hence it is likely that the only disconnections that occur in chromonemas are between half of the newly formed chromioles, before they form a longitudinal connection with their nearest neighbors. In other words, chromonemas may join up from an unjoined state, but do not normally break after having joined.

#### ORIGIN OF CHIASMATA

##### *Division of chromomeres, and formation of new fibers*

For some time after the chromomeres have divided, at mid pachytene, only half the final number of longitudinal connecting fibers are clearly visible in each of the two synapsed chromonemas (as the writer has seen especially in *Lilium*). They are the old fibers. When the new fibers appear between the remaining half of the divided chromomeres (chromioles),

they are at first thin and inconspicuous. But they become indistinguishable from the old fibers at late diplotene (figure 4). Thus when the chromomeres of the two synapsed threads divide each into two chromioles, as they do in mid pachytene, there are only sufficient longitudinal threads for half of them. Each old chromomere forms two new chromioles, and there are no old chromomeres, old chromioles, old chromonemas, or old chromosomes left; only old connecting fibers are visible. Now, these old fibers cannot pass obliquely (as already stated) without an increase in length of about 40 percent. They do not seem to increase in length, but remain directly longitudinal between two of the chromioles (*which* two chromioles is apparently determined by chance). When, after a short time and during late pachytene, the *new* longitudinal fibers start their growth, they are forced



FIGURE 4. Stage (*L. pardulinum*) when the opening-out is just complete (late diplotene, or "diplotene"). Preparation as in figure 1. Viewed below the upper surface. The bivalents form nodes and internodes. A node is not necessarily a chiasma, though many are.

to pass directly longitudinally because of the direct longitudinal position of the old fibers. Hence, there are, normally, no oblique connecting fibers between sister strands (in meiosis or mitosis); and no crossing over, twisting or overlapping of the sister strands.

#### *Overlaps of the two homologous chromonemas*

Overlaps of the two synapsing homologues seem likely to take place often enough to form the usual numbers of chiasmas. They will only form the diagonal X's if the chromomeres are sufficiently apart; for the formation of an X reduces the longitudinal distance between the centers of the chromomeres by about 30 percent. Thus if the centers of the chromomeres are 0.5 micron apart, and the connecting fibers are 0.2 micron long; then an overlap would form an X, with the chromomeres 0.05 micron apart. But if the chromomeres at 0.5 micron distance were connected by fibers only

0.1 micron long, then they could not form the diagonals of an X because these old connecting fibers would not be long enough. In this case the half twist or overlap would perhaps remain more or less external, without causing an X.

The X of the overlap may arise at zygotene with either of two directions of turn. When the chromomeres divide into two, the two diagonals of the X, like the normal direct longitudinal connections between the other chromomeres of the synapsed chromonemas, cannot become oblique, because this would require a growth of about 40 percent over the previous length. Hence they each remain in a plane with one of the two pairs of non-sister chromonemas (halves of synapsed partners). Which of the two planes any diagonal of the X is in, is apparently settled by chance. Thus the two diagonals may remain: (1) in both directions of overlap, together

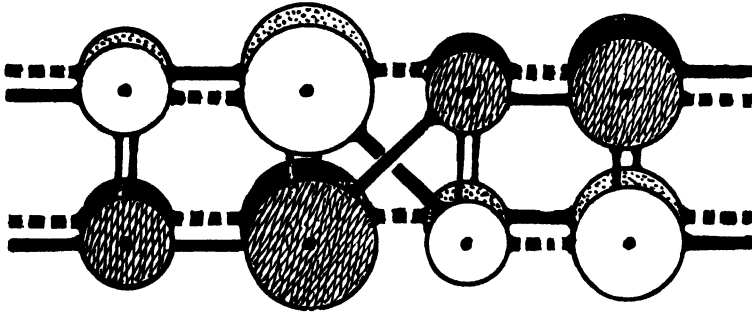


FIGURE 5.—Diagram of a direct chiasma, as seen at pachytene in *Lilium*. The solid lines are the old connecting fibers, the broken lines the new ones.

above; (2) in both directions of overlap, together below; (3) with one direction of overlap, *a* above and *b* below; or (4) with the other direction of overlap, *b* above and *a* below. These four kinds of X will occur equally frequently by chance, only if overlaps in different directions are equal in number. The first two kinds may be classed together as forming *direct* chiasmata (and direct crossovers), and the second two kinds as forming *oblique* chiasmata (and oblique crossovers). The writer has identified both kinds of chiasmata at pachytene in *Lilium*, in about equal numbers.

#### DIRECT AND OBLIQUE CHIASMATA

##### *Direct chiasmata*

Direct chiasmata may occur by chance in half of the overlaps which form chiasmata (this is shown by the study of ANDERSON's genetic results with attached X's, which give equal numbers of direct and oblique chiasmata). When the two diagonals of an X are in the same plane at pachytene (as the writer has observed them sometimes in *Lilium*), then the two pairs of chromioles in the other plane are fairly close together longitudinally

(figure 5), and the new fibers are formed between these chromioles. (We may call the two sister chromonemas of one homologue,  $a$  and  $a'$ ; and those of the partner  $b$  and  $b'$ .) This will give two direct crossover strands,  $a+b$  and  $b+a$  (or the converse), as well as two non-crossover strands, forming the diagonals of the X, namely  $a'$  and  $b'$  (or the converse). If we adopt the convention that the chromonemas  $a$  and  $b$  are above, and  $a'$  and  $b'$  below, we have: with the X above, direct crossovers  $a'+b'$  and  $b'+a'$ , and non-crossovers  $a$  and  $b$ ; and with the X below, direct crossovers  $a+b$  and  $b+a$ , and non-crossovers  $a'$  and  $b'$ .

### *Oblique chiasmata*

Here one diagonal of the X is above and one below, as the writer has sometimes observed in *Lilium* (figure 6), thus forcing the new longitudinal

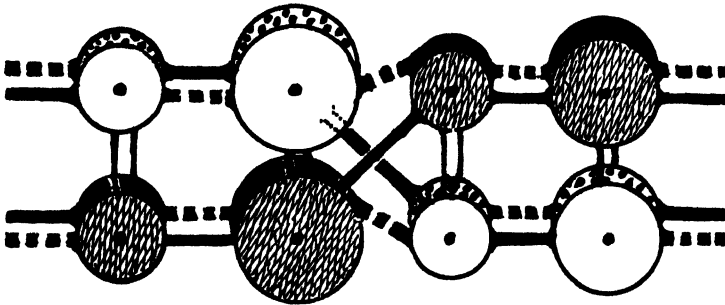


FIGURE 6. Diagram of an oblique chiasma, as seen at pachytene of *Lilium*.

fibers to pass obliquely on each side between the unattached chromioles. (The old fibers of course cannot pass obliquely, because to do so they would have to increase 40 percent in length.) Here also the diagonals do not cross over, and the oblique chromonemas do. There are then two cases (given by the two directions of overlap), in which the top diagonal slants either from upper left to lower right, or from lower left to upper right. Diagonals  $a$  and  $b'$  give oblique crossovers  $a'+b$  and  $b+a'$ , with non-crossovers  $a$  and  $b'$ . With the diagonals  $b$  and  $a'$ , we have the oblique crossovers  $a+b'$  and  $b'+a$ , with the non-crossovers  $b$  and  $a'$ .

### *Adjacent chiasmata*

With two adjacent chiasmata we may have eight cases (and also eight converse cases, made by changing  $a$  to  $a'$ ,  $b$  to  $b'$ ,  $a'$  to  $a$ , and  $b'$  to  $b$ ).

CHIASMATA	CROSSEVERS	
(1) Direct (A)+supplemental direct (A).	$\frac{a+b}{b+a} + \frac{a'+b'}{b'+a'}$	2 similar supplemental chiasmata.
(2) Direct (A)+complemental direct (B).	$\frac{a+b}{b+a} + \frac{a'+b'}{b'+a'}$	2 similar complemental chiasmata.



(3) Oblique (C)+supplemental oblique (C).	$\frac{a+b'}{b'+a} + \frac{a+b'}{b'+a}$	2 similar supplemental chiasmas.
(4) Oblique (C)+complemental oblique (D).	$\frac{a+b'}{b'+a} + \frac{a'+b}{b+a'}$	2 similar complemental chiasmas.
(5) Direct (A)+oblique (C).	$\frac{a+b}{b+a} + \frac{a+b'}{b'+a}$	2 unlike chiasmas.
(6) Direct (A)+oblique (D).	$\frac{a+b}{b+a} + \frac{a'+b}{b+a'}$	2 unlike chiasmas.
(7) Direct (B)+oblique (C).	$\frac{a'+b'}{b'+a'} + \frac{a+b'}{b'+a}$	2 unlike chiasmas.
(8) Direct (B)+oblique (D).	$\frac{a'+b'}{b'+a'} + \frac{a+b}{b+a'}$	2 unlike chiasmas.

The supplemental similar chiasmas produce each two double crossovers and two non-crossovers. The complemental similar chiasmas give four single crossovers each. The four unlike pairs of chiasmas give each one double crossover, two single crossovers, and one non-crossover chromatid.

The four similar pairs of chiasmas have been called compensating, because the jugate chromatids, after crossing over, regularly separate or come together alternately, so that the two (conjunct) threads on one side of an internode adjacent to the left of the first chiasma are continuous with the two threads on one side of an internode adjacent to the right of the second chiasma. So too, the four unlike pairs are sometimes called non-compensating, because the jugate chromatids do not alternate in arrangement, and so two threads on the left of the first chiasma which are on the same side (conjunct) after diplotene will not appear to be conjunct on the right of the second chiasma.

Sometimes the double crossovers in cases of pairs of similar supplemental chiasmas (A+A, and C+C) are called "reciprocal". The double crossovers from the four pairs of unlike chiasmas (A+C, A+D, B+C, and B+D) have been called "diagonal"; but only one chiasma and crossover in each seems diagonal, and C+C is perhaps the truly diagonal chiasma pair.

#### *Chiasmas and crossovers*

Direct and oblique single chiasmas can be shown to be about equally numerous. With regard to pairs of chiasmas, when an overlap in one direction takes place, the next overlap, it seems probable, would occur in the opposite direction; for if it was in the same direction it would cause a twist, which seems unlikely, especially when the ends of the chromonemas synapse first. Hence usually there should be alternation of overlaps, and if every overlap forms a chiasma, there would be an alternation of direction of the overlaps of the diagonals of the X's in sequent chiasmas. This would not affect chance crossing over in direct chiasmas; but with a pair of se-

quent oblique chiasmata the results for crossing over would apparently differ from chance ones. For two sequent oblique chiasmata would most often be complementary rather than supplemental. This would lessen the number of double crossovers expected by chance, for complementary pairs of chiasmata have no double crossovers. If, however, we temporarily disregard this possible source of error, we have by chance 8 pairs of chiasmata occurring with equal frequencies. Having regard to the direction of overlap, we get 32 different pairs of chiasmata; but for the following we may consider only 8 main classes. These give 32 chromatids, and it can be reckoned (table 1) that there are normally, by chance, 8 non-crossovers, 16 single crossovers, and 8 double crossovers, among these.

TABLE 1

CHIASMAS	CHROMOSOMES		
	NON-CROSSEOVERS	SINGLE CROSSEOVERS	DOUBLE CROSSEOVERS
Direct+supplemental direct	2	..	2
Direct+complemental direct	..	4	..
Oblique+supplemental oblique	2	..	2
Oblique+complemental oblique	..	4	..
2 (Direct+oblique)	2	4	2
2 (Oblique+direct)	2	4	2
Totals	8	16	8
Proportion	1	2	1

Thus the proportion for two sequent chiasmata, by chance, is 1 non-crossover, to 2 single crossovers, to 1 double crossover. With a third adjacent chiasma, the proportion would be (1:2:1) (1:1); or 1 non-crossover, to 3 single crossovers, to 3 double crossovers, to 1 triple crossover. For four sequent chiasmata, the chance proportion would be (1:2:1) (1:2:1); or 1 non-crossover, to 4 single crossovers, to 6 double crossovers, to 4 triple crossovers, to 1 quadruple crossover; and so on. (The percentage figures of the crossover chart of a chromosome are made to include the double crossovers, though these do not normally affect the recombinations.) So we have the results given in table 2, for bivalents with terminal fusul chromomeres, or single arms of J or V chromosomes. The distal recombinations are the sums of the odd-crossover chromosomes.

However, it does not seem commonly to happen that the same number of chiasmata occurs in all homologous bivalents. Thus the total chart crossovers may be somewhere between the numbers given in table 2. For the X chromosome of *Drosophila*, the numbers of crossovers in 100 chromosomes have long been published (see MORGAN 1926).

Chromosomes	Percentages	
Non-crossovers	43.5	Total chart crossing over = 70.5
Single crossovers	43	Distal recombinations = 43.5
Double crossovers	13	
Triple crossover	0.5	

Comparing this with the writer's chiasma theory, we note that the distal recombinations are 6.5 below 50 percent. Also the non-crossovers are in excess of the single crossovers, while the opposite happens on the chiasma theory (table 2). Taking the figures as given, we may deduce the following:

	Percentages
No-chiasma bivalents	13
One-chiasma bivalents	37
Two-chiasma bivalents	46
Three-chiasma bivalents	4

TABLE 2

*Chart figures and recombinations in bivalents with terminal fusul attachment.*

NUMBERS OF CHIASMAS	PERCENTAGES OF CROSSEOVERS					CHART CROSSEOVERS	DISTAL RECOMBINATIONS
	0	1	2	3	4		
(0)	(100)	..	..	..	..	(0)	(0)
1	50	50	..	..	..	50	50
2	25	50	25	..	..	100	50
3	12.5	37.5	37.5	12.5	..	150	50
4	6.25	25	37.5	25	6.25	200	50
And so on.							

However, since there is a proximal third or more of the X chromosome without sufficient mutants to detect double crossing over, it is possible that double crossing over is slightly more abundant than found. Perhaps some few distal crossovers are also undetected. No-chiasma bivalents do not seem to occur in attached X's, where their presence could be ascertained (see table 4). Hence, to agree with the writer's chiasma theory (neglecting the rare triple crossovers), the percentage of single crossovers should be increased by 7, and that of double crossovers by 2.

#### ATTACHED AND RING X CHROMOSOMES

##### *Attached X's and crossing over*

In the female *Drosophila*, three of the chromatids of the X bivalent usually pass into the polar bodies. So we cannot normally get both of the crossover chromatids from any crossover. But in the attached X's we regularly get two (non-sisters) of the four chromatids, and these serve to

show the distinction between direct and oblique crossing over. Direct crossovers, in attached X's, are between the two chromosomes of the V; and oblique crossovers are between two V's (but not between sister chromatids). If the attached X's are originally heterozygous for several loci, then single direct chiasmata do not change this heterozygosity: but single oblique chiasmata (that is  $1/2$  of the single chiasmata) make all heterozygous loci distal to them homozygous in the resulting attached X's. That is, if all chiasmata are single, 50 percent of the attached X's (if heterozygous originally) are homozygous distal to the chiasmata. But, with all chiasmata single, 50 percent of the resulting chromosomes show one crossover, and 50 percent are non-crossovers. Hence the percentage of single-crossover chromosomes from single chiasmata is equal to the percentage of distally homozygous attached X's from single chiasmata. The percentage of *recessive* distally homozygous attached X's is half this (or half the percentage of single crossovers from single chiasmata).

Of the eight kinds of double chiasmata (table 1), two, both proximal direct plus distal oblique, give distal homozygosity of originally heterozygous attached X's. Of the 16 resulting attached X's, two are homozygous for recessives; that is, 12.5 percent. These 16 attached X's have 32 chromosomes, of which 8 (on the average) are non-crossovers, 16 are single crossovers, and 8 are double crossovers. This would yield a crossover chart total of 100 percent crossing over. It gives 25 percent double crossovers. Thus the percentage (due to double chiasmata) of attached X's distally homozygous for recessives is half the double crossovers (or, a quarter of the total chart single crossovers due to double chiasmata).

Now the crossovers in the X of *Drosophila melanogaster* may be taken as (excluding the rare triple crossing over, and also the possibility of no-chiasma bivalents) nearly equal to,

Non-crossovers, 35 percent	Total chart crossing over = 80.
Single crossovers, 50 percent	
Double crossovers, 15 percent	Distal recombinations = 50.

These would arise from:

Single chiasmata, 40 percent
Double chiasmata, 60 percent

The distal recessive homozygosity for 100 resulting attached X's would be half the crossovers from single chiasmata,  $20/2 = 10$  percent, plus half the double crossovers,  $15/2$  or 7.5; totaling 17.5 percent. The proximal parts of the attached X's, with about 10 percent of chart crossovers, should give about  $10/2$ , or 5 percent of recessive homozygosity; since there are few or no proximal double crossovers to be reckoned with here.

Since the total chart crossovers are made up of the total single crossovers ( $s$ ) plus twice the double ( $d$ ) crossovers (that is,  $s + 2d = C$ ), the recessive distal homozygosis is  $\frac{s-2d}{2} + \frac{d}{2} = \frac{s-d}{2} = \frac{50-15}{2} = 17.5$ ; or  $= \frac{C-3d}{2} = \frac{80-45}{2} = 17.5$ . STURTEVANT'S value for the *sc* locus is 17.1. The higher value of 19 for the locus *y* is probably due to differential viability (STURTEVANT 1931).

The values for *f* and *g* in table 3 (calculated from single chiasmata only) are over the values found. (It seems possible that there were a few double chiasmata proximal to these.) The data do not permit of the calculation of the values from *m* to *ec*.

TABLE 3

MUTANT LOCI	PROXIMAL CROSS- OVERS FOUND	PERCENTAGES OF DISTALLY HOMOZY- GOUS RECESSIVE AT- TACHED X'S FOUND	PERCENTAGES CAL- CULATED FROM PROXIMAL CROSS- OVERS AND CHIASMAS	
<i>f</i>	13.5	5.1	6.8	Mainly single crossovers
<i>g</i>	26	10.3	13.0	
<i>m</i>	33.9	13.5		Single and double cross- overs
<i>v</i>	37	14.8		
<i>i</i>	42.4	16.1		
<i>ct</i>	50	16.4		
<i>cv</i>	56.3	15.9		More double crossovers
<i>ec</i>	64.5	16.5		
<i>sc</i>	70	17.1	17.5	
<i>y</i>	70	(19)	17.5	

For ANDERSON'S important experiments (1925), the attached X's may be divided into 3 regions: (A) from the fusul (spindle) chromomere to the locus of *f*, chart distance = 13.5; (B) from *f* to *ct*, chart distance = 36.5; and (C) from *ct* to *sc* (presumably near the distal end), chart distance = 20. In A the double crossovers are apparently unknown; in B they are ascertained; and in C they have not been measured in this experiment. However, we have assumed, for the X chromosome, that the total double crossovers are 15 percent. Those with both or the second crossovers in B are 5 percent. Thus 10 percent have their second crossovers in C. (Hence we can remove the second of the double crossovers from the chart distance in B and C, and consider only the proximal crossovers.)

Of the 13.5 single crossovers in A, 6.75 are probably oblique and produce homozygosis; and 6.75 would be direct. Taking the total double crossovers in B and C as 15, we may subtract the second (distal one) of these in

sections B and C; B having 5 known, and C therefore having the remaining 10.

TABLE 4

SECTION	CROSSTERS IN ATTACHED X's	ATTACHED CHROMOSOMES AND CHIASMAS
A	13.5	$\left\{ \begin{array}{l} \text{Direct, complementary non-crossovers, 6.75} \\ \text{Oblique, identical non-crossovers, 6.75} \end{array} \right.$
B	$36.5 - 5 = 31.5$	$\left\{ \begin{array}{l} \text{Direct, 15.75 } \left\{ \begin{array}{l} \text{complementary crossovers, 7.88} \\ \text{complementary non-crossovers, 7.88} \end{array} \right. \\ \text{Oblique, 15.75 } \left\{ \begin{array}{l} \text{crossovers and non-crossovers} \\ \text{non-crossovers and crossovers} \end{array} \right. \end{array} \right. 15.75$
C	$20 - 10 = 10$	$\left\{ \begin{array}{l} \text{Direct, } \\ \text{Oblique, } \end{array} \right\} \text{complementary non-crossovers, 10}$

Thus we have the totals of attached X's:

- (1) Complementary non-crossovers  $= 6.75 + 7.88 + 10 = 24.63$
- (2) Identical non-crossovers  $= 6.75$
- (3) Crossover and non-crossover  $= 15.75$
- (4) Complementary crossovers  $= 7.88$

From this we get table 5. The fit would be somewhat closer if one or

TABLE 5

	CALCULATED FROM CHART DISTANCES	ANDERSON'S RESULTS FROM ATTACHED X's
(1) Complementary non-crossovers	44.8	43.9
(2) Identical non-crossovers	12.3	10.8
(3) Crossover and non-crossovers	28.6	29.7
(4) Complementary crossovers	14.3	15.6

more of the double crossovers was found to be included in the 13.5 chart distance from *f* to the proximal end; for the chief difference is in the 5.4 percent of oblique crossovers found proximal to *f*, giving 10.8 (oblique + direct) crossovers; whereas the crossover chart has 13.5 crossovers here.

### *Ring X's and crossing over*

L. V. MORGAN (1932, and *in litt.*) showed that when a ring X synapses with another ring X (or with a rod X) double-crossover X chromosomes survive, but no single crossovers appear in the progeny. Two chromonemas crossing over once would form one large ring (or one long rod) with two fusel chromomeres. This does not survive in the progeny. Double crossovers would form two rings (or a ring and a rod) and survive. But two

sister chromonemas crossing over would also form one inviable large ring (or long rod). If sister-strand crossing over occurred, the numbers of non-crossover chromosomes would be diminished with regard to the numbers of double-crossover chromosomes. This is not the case. Therefore sister-strand crossing over does not occur (normally) between two ring X's (or between the ring and the rod X). Hence it was not postulated above to solve the problem of attached X's, nor would it fit these. Therefore it probably does not occur in normal X's.

#### EXPLANATION OF GENE REARRANGEMENT

##### *Reversed crossing over*

This appears to originate usually at meiosis, and in a normal trisomic plant (*Datura*); but less commonly in a diploid (1927). On the writer's theory, if the fusul chromomeres synapsed, but the rest of the two chromonemas was in reverse order, crossing over could take place only close to the fusul chromomeres. Such reversed crossing over would give two chromosomes; one with two left arms, and the other with two right arms. The third homologous chromosome, in the trisomic plant, might help this process by synapsing first with one or both ends of one of the chromonemas concerned. Reversed crossing over in *Datura* appears to have occurred only next the fusul chromomere.

##### *Reciprocal translocation*

If any two heterologous chromonemas overlapped close enough when their chromomeres were first dividing, there might be (by the writer's theory) cross-connections found between two of the four, as in an ordinary chiasma (but not between sisters). Such cross-connections would produce reciprocal translocations, in which the interchanged parts were neither equal nor homologous. A bend at the point of overlap might result in a gene or more being omitted at the point of interchange.

It has been suggested that heterologous interchange results from interlocking of bivalents at meiosis. This seems possible; but it is also possible that some cases styled "interlocking" at meiosis may be due to interchange. If interchange occurs at *mitosis*, it cannot apparently be due to interlocking.

##### *Terminal translocation*

The writer has seen, in meiosis, the ends of the chromonemas resting laterally on other chromonemas. If cross-connections like those at a chiasma occurred during longitudinal divisions, chromonemas would result with terminal attachment of a heterologous segment. This might also occur at mitosis.

*Inversion and deletion*

If a dividing mitotic chromonema (or a bivalent at early pachytene) overlapped itself closely in a loop, when its chromomeres divided there might be formed cross-connections between two threads, as in a chiasma. This would result in an inversion (or a deletion). The deleted piece may form a ring (McCLINTOCK 1933). It is readily seen how a gene might be lost in the inversion, by the connecting fiber passing it by.

*Insertion*

This is perhaps a double interchange, where a loop of one chromonema overlaps another chromonema at two near places. The piece lost may be small. Insertion should be quite rare, since it requires two rare interchanges to cause it.

*Terminal deficiency*

This seems common. There are several possible ways in which it may arise. Every terminal translocation, for instance, is accompanied by a terminal deficiency in another chromonema.

## OBJECTIONS TO THE THEORY

The following objections have lately been made to the present writer's modified Janssens' theory of crossing over; which accounts also for translocation, deficiency, interchange, inversion, deletion, and insertion.

*First Objection.* That direct chiasmata formed at overlaps, according to the writer's theory, can at most result in 50 percent of chromatids showing crossing over; whereas some chromosomes of *Drosophila* actually show a greater percentage than this. *Reply.* If the two pairs of sister chromatids at crossing over are  $a$  and  $a'$ , and  $b$  and  $b'$ , respectively; and  $a$  is opposite to  $b$ , and  $a'$  is opposite to  $b'$ ; then, at the overlaps, direct chiasmata may be formed in which  $a$  crosses over with  $b$ , and also by chance, in equal numbers direct chiasmata in which  $a'$  crosses over with  $b'$ . So the four chromatids from a bivalent which has more than one direct chiasma, may sometimes all be crossovers; and it is possible that, in some cases of long chromosomes, the limit of 100 percent of crossover chromosomes may be reached. (Oblique chiasmata are left out of consideration here; but would, of course, add to the numbers of crossover chromosomes.)

*Second Objection.* That, on the writer's theory, the pairs of sister chromatids formed by the secondary split at meiosis should show half as many apparent twists as there are genes in the chromosome; and the same should happen between the two halves of a split chromosome in any mitotic division. *Reply.* It is a fact of observation that the longitudinal connection fibers between chromioles pass the shortest way (GELEI 1921, BELLING 1931a). The shortest way between any two homologous chromioles of a split chromonema and their two neighbors on either side is not obliquely



but longitudinally. Hence the old fiber will be longitudinal, and so force the new one to be so. Thus, also, there will be (normally) no twists between sister strands, though there may be rarely twists of the whole chromosome or bivalent. The difficulty with the objector, and this holds also for the first objection, is that there is no *old* chromiole when a chromomere has divided. The *old* chromomere has gone, and there are two new chromioles formed, which are equivalent. So also the old chromonema when divided changes into two new chromonemas. (There are, however, old connecting fibers, and subsequently new ones also, between adjacent chromioles.)

*Third Objection.* That there is always or usually a telophasic split in somatic divisions, and also that the leptotene of meiosis is always or usually split. *Reply.* A telophasic split has been predicated mainly by observers who have used sections of plant (or animal) material, fixed *en masse*. This method, in the writer's opinion, does not show fine nuclear details truly, but gives the alveolar artifacts of slow fixation. This is proved by comparison with similar material made into smear preparations by the best methods (see BELAR 1929a, 1929b). The writer has spent some months with smear preparations of mitosis (both diploid and haploid), especially of *Tradescantia* and *Rhoeo*, and has convinced himself that there is no telophasic split visible in his material. He is also satisfied that there is no longitudinal split to be seen, with good microscopy, in the leptotene threads of the liliaceous plants he has examined.

*Fourth Objection.* That bivalents with unequal homologues are inconsistent with the writer's theory of crossing over. *Reply.* It was the behavior of such bivalents which first led the writer to discredit the alternate-opening-out hypothesis of the origin of chiasmata. The effects of opening-out in these bivalents are visible at early diaphase. They do not show, in the cases examined, opening-out of the sister chromatids at the unequal free ends. They should show this in about half the cases, on the alternate-opening-out hypothesis, when sliding of the X's of chiasmata had not taken place. In WENRICH's excellent figures of *Phrynotettix* (1916), all the bivalents with unequal homologues show at early diaphase that the unequal extremities have not opened out at the secondary split. The writer has observed this same fact in a bivalent of *Aloë* with unequal homologues.

At the first anaphase, with a terminal (or sub-terminal) fusel point as in *Phrynotettix*, if there is no crossing over, or if there are two similar supplemental chiasmata, then the two long chromatids separate from the two short chromatids and pass to opposite poles (as in some of WENRICH's figures). But if there is one chiasma, a long and a short chromatid pass together to each pole (as in other figures of WENRICH). The explanation of this is that there has been crossing over. The alternate-opening-out hypothesis of chiasma origin apparently cannot apply here, as already shown.

*Fifth Objection.* That the reduction in the number of nodes observed in some plants, from early diplotene to metaphase, is due to the breaking and rejoining of chiasmata; not to the disappearance of some loose twists, or overlaps, or of temporary lateral fusions. *Reply.* Since the nodes (apparently mostly temporary chromatin cross-connections) at early diplotene in *Allium triquetrum* seem somewhat over 200 in number (1931a), while the chiasmata at diaphase may be about 20, a loss of about 180 nodes results. It does not seem probable that these many lost nodes were chiasmata. For nodes are stated not to be lost in *Stenobothrus* from late diplotene to late diaphase (DARLINGTON and DARK 1932). Nor should it be assumed without proof that a node is a chiasma. That chiasmata break and join is improbable, for the writer knows no case of chromosomes joining after proved fracture. On the writer's hypothesis there is normally no fracture in crossing over, translocation, interchange, inversion, deletion, deficiency, or insertion.

*Sixth Objection.* If non-disjunction of the X chromosomes in *Drosophila* is attributed to the X's having so many chiasmata that they fail to disjoin; then, in the high non-disjunction line, why do the X's show less crossing over instead of more? *Reply.* The first part of this objection is a conjecture. Since the high non-disjunction is admittedly due to translocation or inversion, it seems probable that the apparent non-disjunction is sometimes non-conjunction, as the writer has noted in triploids and tetraploids (and also in *Uvularia*). If there are cases of whole or partial non-conjunction or asynapsis (compare McCLINTOCK 1931), then the percentage of crossing over would be decreased.

*Seventh Objection.* The opening-out of the X of a chiasma towards the ends of the bivalent must separate the two chromosomes at this end, instead of giving a terminal junction. *Reply.* Since the terminal chromomeres visibly hold the leptotene-zygotene chromonemas together at the distal ends in a number of organisms, it is not improbable that the same distal chromomeres may hold the four jugate chromatids together when brought into contact at the ends. In this case the opening-out of the X of a chiasma at diplotene when it reaches the end of a bivalent will bring the terminal chromomeres of the chromatids into close proximity, and connection by new cross threads may result.

*Eighth Objection.* That the percentage of homozygosis of recessive alleles that were heterozygous in the parent, towards the distal (left) end of the attached X's of *Drosophila*, was found by ANDERSON, and also by others, to be slightly more than that calculated for random assortment of the recessive alleles. This was said to be inexplicable on the writer's hypothesis. *Reply.* The writer's hypothesis gives different grades of homozygosity of recessive alleles, according to the amount crossing over from proxi-

mal single chiasmata, equalling half of these crossovers, since only oblique (not direct) chiasmata cause homozygosis of heterozygous alleles in attached X's. There must be added to this half the double crossovers. For the X chromosome, terminal recessive homozygosity is about 17.5 percent on the writer's theory.

*Ninth Objection.* In the translocation of a terminal piece of one arm of the third chromosome to the fourth chromosome of *Drosophila*, crossing over was less in the proximal part of the attached portion of the third chromosome, in flies homosomic for the translocation, than in the normal flies. This was said to be inexplicable on the writer's theory. *Reply.* If synapsis begins with the small fourth chromosome in the combined piece (as it should, since the fusal point of the third chromosome is left behind), then there will be interference between this point and the first chiasma, and few crossovers will occur near this end; as perhaps happens also near the proximal end of the X, and near the fusal points of the second and third chromosomes (see also DOBZHANSKY 1931).

*Tenth Objection.* In haploid *Zea*, a split was found in the threads of the meiotic prophase. It was objected that this was contrary to the hypothesis of the writer, which is based on observations of an unsplit leptotene. *Reply.* The leptotene stage is more difficult to demonstrate than the pachytene, especially in aceto-carminic smears. The pachytene stage of the diploid *Zea* has been figured and photographed by McCLINTOCK; but good figures and photographs of the leptotene are yet to be sought. The writer would suggest that further work might perhaps show a stage in the haploid *Zea* comparable with the leptotene of the diploid, as the split stage found in the haploid is perhaps comparable with the late pachytene of the diploid *Zea*.

*Eleventh Objection.* That a tertiary split has been demonstrated in the pachytene of one plant, and also that there were two split spiroid chromonemas in the first anaphase chromosomes. That this is contrary to the writer's hypothesis, in which the pachytene has only two splits, and the first anaphase chromosomes show two unsplit chromonemas. *Reply.* When the late pachytene is well enough fixed to show the chromomeres distinctly, only two splits are visible in *Lilium* and *Fritillaria*. Preparations which are to demonstrate a hitherto invisible tertiary split should perhaps show the chromomeres as distinctly as those obtained by the writer.

The spiroid threads found in the metaphase and anaphase chromosomes by appropriate destaining may perhaps appear with lighter centers when their chromatin is disappearing, as is the case from anaphase to telophase. Most workers however have found that the first-anaphase spiroid threads are only two in number. That this is the case in *Rhoeo* and *Tradescantia*, the writer also can testify, from iron-brazilin and iron-haematoxylin smears, fixed with Navashin's or with Flemming's mixture.

*Twelfth Objection.* That the genetic proof (PATTERSON 1933) of the chromosomes in the sperms of *Drosophila* being probably sometimes split, furthers the hypothesis of a telophasic split, and so is against the writer's hypothesis. *Reply.* The dense accumulation of chromatin in the sperms of most animals and some plants usually hides the state of the chromonemas. But when the sperm nucleus spreads out in the fertilized ovum, in plants and animals, it is usually found to be in the later prophase, and at a stage when the chromosomes are normally split. Hence the splitting of chromonemas may possibly take place in the spermatozoön. In a certain nematode, according to MULSOW, the chromosomes in the sperm are at the metaphase stage, and doubtless already split. PATTERSON's results also show that the chromonemas in the sperm are sometimes (in six-sevenths of the cases) *not* split, which is contrary to the "telophase-split" hypothesis of some writers.

*Thirteenth Objection.* That the chromomeres found in the pachytene of diploid *Zea* (by the acetic-alcohol and aceto-carminc method) do not correspond in the two synapsed threads. *Reply.* These "chromomeres" are often compound chromomeres run together during fixation, and then may be composed in different ways in the two threads. The same thing happens in *Lilium*, *Fritillaria* and *Aloë*, if the fixation is not rapid enough.

*Fourteenth Objection.* That no crossing over between sister chromatids was found either between two ring X's or between a normal and a ring X, in *Drosophila*. *Reply.* Sister-strand crossing over was, because of this fact, excluded from the writer's theory, which now uses equal numbers of direct and oblique chiasmata only, as calculated from ANDERSON's results with attached X's, and confirmed by observation of the pachytene of *Lilium*.

*Fifteenth Objection.* That the writer's theory does not fully explain ANDERSON's results with attached X's and equational exceptions. *Reply.* In the first form of the writer's hypothesis these could be only approximately explained by diagonal and sister-strand assortment at the spindle-fiber end. The present form of the theory enables ANDERSON's numerical results to be calculated with fair accuracy (see above).

*Sixteenth Objection.* That the late pachytene of *Zea* shows more nodes to a chromosome than the chiasmata seen at diaphase. *Reply.* A chiasma presumably arises only at an overlap, which is a kind of half-twist with no rotation in each chromonema. If the chromomeres are too close together, an overlap or half-twist may not result in a chiasma. Also at earliest diplotene, besides chiasmata, there are many (often hundreds of) chromatin connections across the primary split. A few of these may remain and hold the threads together for a time.

*Seventeenth Objection.* Since the action of X-rays of definite frequency in causing point mutation is taken as indicating that the bare genes are too

small to be visible with the microscope, therefore the genes cannot be directly counted. *Reply.* The writer's observations in destaining ultimate chromomeres in hyrax have shown that each contained one minute stainable central body (like a centriole). The cells were examined with the monobromide of naphthalin objective, at a working aperture of 1.5. The central particles were probably over 0.07 micron across. They were doubtless covered with chromatin. The bare gene is then smaller than this. Hence, also, with regard to mutation, since the gene substance in one chromomere is not divisible by crossing over, this must be regarded as a whole; that is, as one gene. Whether the whole, or only part, of a gene is acted on by the X-rays does not seem generally ascertained. But in the formation of multiple alleles the action must apparently be partial. In this case the size of the whole gene does not seem deducible from the (direct or indirect) action of X-rays on a part of it.

*Eighteenth Objection.* It has been estimated that *Drosophila melanogaster* has over six times as many genes as the writer found in *Lilium*, and as seem to be about the number of ultimate chromomeres indicated in the grasshopper *Phrynotettix* (WENRICH 1916). *Reply.* This estimation seems to the writer to mingle point mutations with gene rearrangements (deficiencies, inversions, interchanges) as causing dominant or recessive lethals. It also does not allow for the sperm chromonemas having sometimes split especially in the autosomes. Hence the resulting figure is too high (see PATTERSON 1933, McCLINTOCK 1931).

#### SUMMARY

1. The writer's modification of JANSSENS' hypothesis explains crossing over, and also explains gene rearrangements, such as reversed crossing over, reciprocal translocation, inversion, deletion, and deficiency.
2. The chromonemas were proved to be unsplit at leptotene in certain plants.
3. Living (and also fixed) chromonemas of resting "final" nuclei showed no split, in the plants examined.
4. The secondary split was first seen at mid pachytene.
5. Both direct and oblique chiasmata were seen at pachytene in *Lilium*.
6. After the chromomeres have split, the old longitudinal fibers are either alone visible; or are seen to be thicker than the new ones.
7. In *Lilium* the opening-out at diplotene seems to be only at the primary split. (However, in plants such as *Datura*, with no chiasmata at diaphase, it is probable that the diplotene opening-out alternates at the chiasmata.)
8. Since chiasmata arise at pachytene in certain liliaceous plants, they cannot arise from alternate opening-out at diplotene.

9. Chiasmata seem to be due to overlaps, not twists. Overlaps may be sometimes mistaken for twists, under the microscope.

10. There are 8 main kinds of double chiasmata, equally numerous by chance.

11. Double chiasmata give, by chance, one non-crossover chromosome, two single-crossover chromosomes, and one double-crossover chromosome.

12. If crossovers arise from chiasmata, then the distal recombinations from the end to the fusel chromomere should be 50 percent.

13. If crossovers arise from chiasmata, then the chart crossovers divided by 50 should give the average number of chiasmata.

14. The ascertained numbers of crossover X chromosomes of *Drosophila melanogaster* appear to lack about 7 percent of single crossovers, and about 2 percent of double crossovers, if they arose from chiasmata (neglecting triple crossovers).

15. Flies with heterozygous attached X's in *Drosophila* (ANDERSON 1925), should (on the writer's theory) give distal recessive homozygotes in a percentage equal to half the chart length minus one and a half times the percentage of double crossovers. This would be 17.5.

16. On the writer's theory, the percentages of complementary and identical non-crossovers, of crossovers plus non-crossovers, and of complementary crossovers, in attached X's, have been calculated from the chromosome chart, and agree with ANDERSON's experimental results.

17. L. V. MORGAN's work with ring X's in *Drosophila*, like ANDERSON's results with attached X's, shows the absence of sister-strand crossing over. Therefore chiasmata formed by a half twist between the two homologues are absent, or rare (1931b).

18. It is possible to explain reversed crossing over, heterologous interchange, terminal translocation, inversion, deletion, and deficiency, by the overlapping of two chromonemas when their chromomeres are dividing. The result is equivalent to the formation of a chiasma between synapsed homologues; but is less regular, so that genes may be lost at the junctions.

19. Some objections to the writer's theory are briefly answered.

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# ON THE GENETIC CONSTITUTION OF JERSEY ~~CAT~~ TLE, AS INFLUENCED BY INHERITANCE AND ENVIRONMENT<sup>1</sup>

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The high class dairy cow has taken its present form first because of its survival value in nature and second because of centuries of controlled breeding. In both instances the constitution toward which the breeding tended was approached without conscious knowledge of how inheritance was achieved. It was a case of evolution rather than exact breeding based on genetic factors and a known method of inheritance. Yet, after all, this is the way in which man and most of his economically important animals and plants evolved. It is significant to take such a population, analyze it for the mode of inheritance of the characters involved, and amount of heterozygosis which is now extant within the population, determine present distribution of this inheritance, and from this information formulate a picture of the forces which have been at work to guide the animal of today to its present form. The data of this paper and their analyses are presented as a contribution to the quantitative interpretation of this problem as far as it relates to Jersey cattle.

The Island of Jersey lies in the English Channel, but a few miles from the French mainland. There is little doubt but that the remote ancestors of the Jersey breed of cattle were identical with the common stock found on this neighboring mainland, where cattle of similar form and color can still be seen. In the 18th century the cattle of this island, not 30 miles around, were set apart from those of the rest of the world by laws prohibiting the importation of any cattle. These laws have been in force since 1763, or for about 40 generations in the life scale of cattle. The Royal Jersey Agricultural Society has systematically tried to standardize the animals by drawing up scales of points and guiding the breeding operations of the individual farmers. The Jerseys of America were imported from this island stock, most of the importations being from the latter quarter of the 19th century to the present day. The million or so progeny from these imported cattle have been kept separate from the other cattle by registration of pure breeding. They have become distributed over the whole United States.

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A population of 6000 animals scattered over 15 States will obviously vary in heredity and environment, feeding, care, et cetera. If the conformation of these animals is dependent on the variations of inheritance which have been available to such groups, then the parent and offspring and fraternal correlations will furnish a measure of the effect of inheritance. Similarly, if the variations of environment are to be factors in the determination of the size of these animals, then the uniformity of the animals within one herd, as contrasted with those in another, as determined from the proper correlations, measures this effect. But both measures have some part of the other variable within them. Parent and offspring correlations are, in the main, freed from the influences of common environmental variations since years elapse between the growth period of the parent and that of the

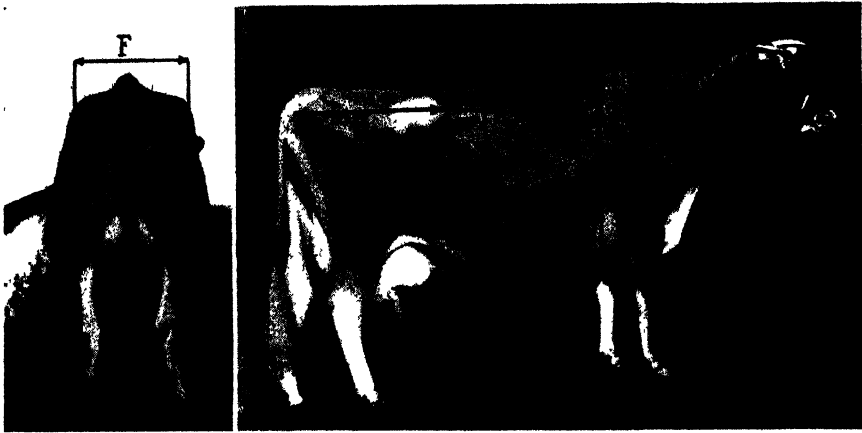


FIGURE 1.—Photographs of Jersey cows showing the points used in making the measurements studied herein.

offspring. This is especially true where the parents and offspring are reared in different herds. The fraternal correlations are not so one-sided a measure of the inheritance as they include also the environmental element of common rearing. The possible influence of selective breeding based on the external appearance of the animal because of its effect on the ancestral correlations enters into this comparison. These questions may be approached through the use of the excellent data on the American Jersey Cattle. (The American Jersey Cattle Club Directors, realizing that the future of any coordinated effort in dairy cattle breeding depends largely on accurate, well analyzed knowledge of all phases of the problem, have had collected the body of information herein described.)

These data include the following measurements on the bull's or cow's type: estimated weight, height at withers, heart girth, paunch girth, width at hips, body length and rump length. Figure 1 shows the relative position

of the measurements taken. About 300 bulls and 6000 cows were measured. The animals come from herds in 15 different States distributed quite generally throughout the United States. Two capable, experienced dairy cattle men took all of the measurements; one man, Mr. RANDOLPH, took all but about 800. The ages, parentages, et cetera of these animals are known through their registration in the herd books. All of the measurements utilized for this study have been age corrected so that all records are strictly comparable for this variable. The volume of material is sufficient to cover the immediate questions: (1) The relation between the sire's conformation and that of his daughters; (2) The correlation between the dam's conformation and that of her daughters; (3) The relation between the types of the full or half sisters; and (4) The assortive mating between sire and dam. (For the study of these data from the viewpoint of conformation and its relation to milk yield et cetera see the literature list (1).)

#### THE RELATION BETWEEN THE SIRE'S CONFORMATION AND THAT OF HIS DAUGHTERS

For this comparison, there are available 736 pairs of sires and their daughters, some of the pairs being formed by the same sire with different daughters. If the type of the sire is transmitted to the daughter it would be expected that the daughter would resemble the sire's type. By contrasting the measurement of the sire with that of his daughters, it is possible to determine the degree of relationship which exists between their types. The amount of this relationship will be due primarily to the cow's inheritance and secondarily to her environment (such as nutrition, care, et cetera) Table 1 shows the average daughter's size for given conformational measurements of the sires.

The comparison of the sire's type with that of his daughter as shown in table 1 indicated that there is a direct relation between the sire's size and his daughter's conformation. The larger the sire in any given conformational measurement, the larger his daughters tend to be. Thus, sires with a weight of 1025 pounds have daughters whose average weight is 725 pounds, and sires whose weight is 1575 pounds have daughters whose average weight is 1030 pounds. The results, however, are quite irregular, showing that the relation is not a close one. A similar relationship may be drawn for the measurements of sire and daughter in height at withers, depth at withers, heart girth, and paunch girth.

The irregular line of figure 2 represents the actual average daughter's measurements for the given sire's measurements shown in table 1. The straight line shows the general average trend of these measurements. The heavy portion of the line represents the average daughter's measurements which are based on more than 10 individuals.

TABLE 1  
*Relation between sires' conformations and those of their daughters.*

WEIGHT*		HEIGHT AT WITHERS*		DEPTH AT WITHERS		HEART GIRTH		PAUNCH GIRTH		WIDTH AT HIPS		BODY LENGTH		RUMP LENGTH	
SIRE'S	DAUGHTERS' AVERAGE	SIRE'S	DAUGHTERS' AVERAGE	SIRE'S	DAUGHTERS' AVERAGE	SIRE'S	DAUGHTERS' AVERAGE	SIRE'S	DAUGHTERS' AVERAGE	SIRE'S	DAUGHTERS' AVERAGE	SIRE'S	DAUGHTERS' AVERAGE	SIRE'S	DAUGHTERS' AVERAGE
775	870	47.5	47.0	26.5	26.5	67	70.0	77	83.0	16.5	18.5	31.0	35.7	19.5	19.9
975	685	48.5	46.7	28.5	27.3	71	67.0	79	86.6	17.5	19.2	33.0	36.3	20.5	19.8
1025	725	49.5	47.2	29.5	27.0	73	69.8	81	82.0	18.5	19.4	35.0	36.2	21.5	20.2
1075	970	50.5	47.6	30.5	27.3	75	69.5	83	85.3	19.5	19.7	37.0	—	22.5	20.6
1125	895	51.5	47.3	31.5	27.7	77	70.0	85	87.0	20.5	20.1	39.0	36.1	23.5	21.5
1175	885	52.5	48.3	32.5	27.8	79	70.5	87	85.1	21.5	19.9	41.0	37.0	24.5	21.6
1225	900	53.5	48.2	33.5	27.1	81	72.0	89	87.3	22.5	21.5	43.0	38.4	25.5	21.8
1275	910	54.5	48.1	34.5	27.6	83	71.9	91	87.2	23.5	21.5	45.0	39.2		
1325	895	55.5	49.7	35.5	28.7	85	71.2	93	89.4	24.5	21.1	47.0	40.5		
1375	930	56.5	51.0	37.5	28.8	87	74.2	95	85.8						
1425	925	58.5	48.5			89	70.6	97	88.2						
1475	940							99	89.0						
1525	950							101	89.7						
1575	1030							103	91.6						
1625	970							105	87.9						
1675	1000							107	88.4						
1725	925														

\* Weight in pounds, other measurements in inches

These results clearly demonstrate a relation between the size of the sire and the size of his daughter. The relation, however, is not very exact, allowing a fairly large variation in either direction. Two factors in the cow's

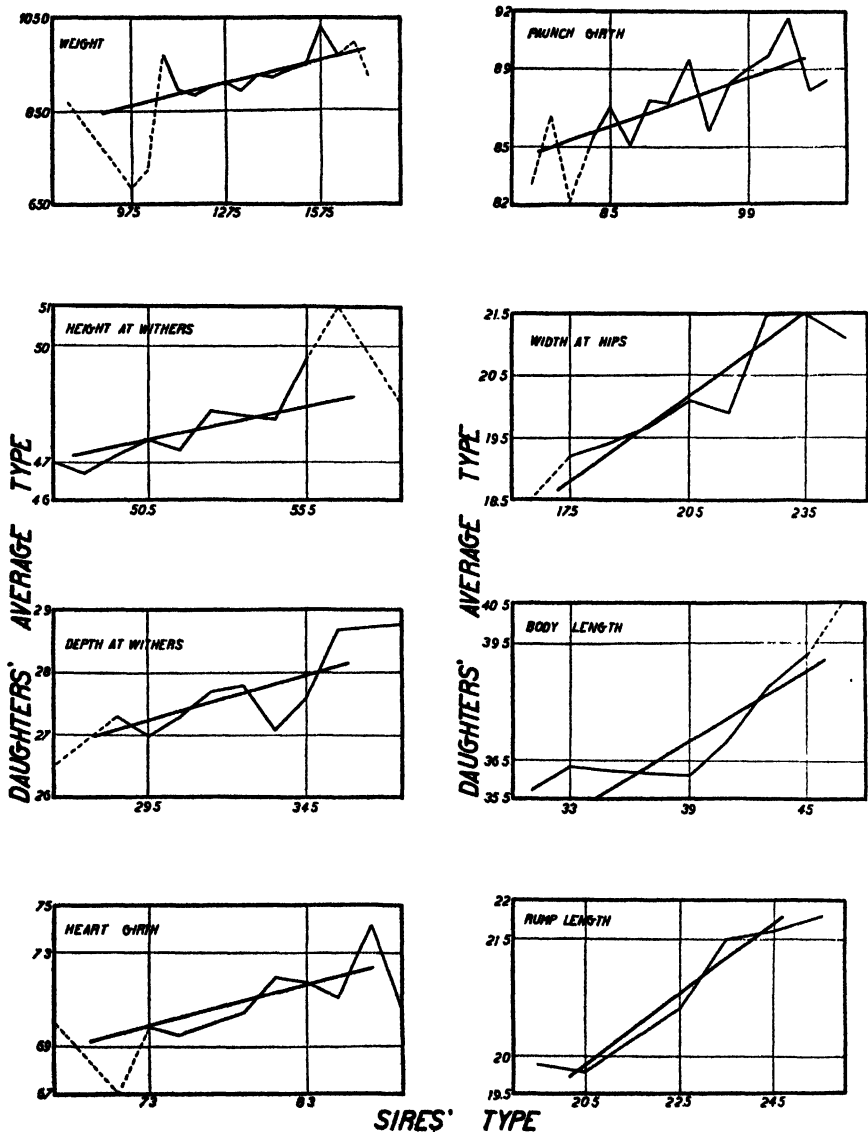


FIGURE 2.—Relation between sire's size and daughter's size in Jersey cattle.

development may bring about such a resemblance between the sire and his daughter: the first is the heredity for size which the sire transmits to his daughter; the second is the condition such as feeding and care which surround each, tending to make them resemble each other. In view of the fact that most sires are grown in other herds and are purchased at an age

when it would be difficult to influence their size, it seems fair to interpret these correlations as due to heredity. The correlations show that the breeder of dairy cattle may reasonably expect his choice of type in the sire to tend to produce daughters which will themselves resemble the sire's type to some degree. As the correlations are quantitatively not high, the residual or uncontrolled variation which would be expected in the type of such daughters would still be large.

If the sire's conformation for one given body measurement, say, height at withers, is compared with the body measurement of the daughter for perhaps body length, it is found that here too there is an intimate relation between the sire's size and the daughter's size. In other words, if the sire selected is of large conformation in a given body part, he tends to beget a daughter of large conformation for other given parts.

The correlation coefficients and prediction equations on which the above conclusions are based are found in table 2.

TABLE 2  
*Relation between the measurements of the sire and the measurements of his average daughter.*

SIRE'S	DAUGHTER'S									STANDARD DEVIATION
	WEIGHT	HEIGHT AT WITHERS	DEPTH AT WITHERS	HEART GIRTH	PAUNCH GIRTH	WIDTH AT HIPS	BODY LENGTH	RUMP LENGTH	SIRE'S AVERAGE SIZE	
Weight	.34	.33	.27	.29	.31	.34	.39	.29	1399±11.4	164±8.1
Height at withers	.29	.31	.33	.35	.33	.38	.30	.31	52.2±.14	2.0±.10
Depth at withers	.14	.19	.29	.28	.38	.38	.34	.32	32.0±.12	1.7±.08
Heart girth	.11	.13	.17	.30	.38	.35	.36	.33	80.4±.26	3.7±.18
Paunch girth	.08	.06	.12	.18	.31	.32	.34	.24	93.1±.46	6.6±.32
Width at hips	-.01	.07	.09	.32	.32	.65	.44	.59	21.1±.12	1.7±.08
Body length	.04	.22	.18	.28	.42	.35	.42	.33	44.3±.25	3.0±.17
Rump length	.10	.12	.08	.37	.34	.57	.43	.51	22.5±.10	1.4±.07
Daughter's size	933 ±3.0	47.8 ±.04	27.6 ±.03	71.2 ±.09	87.5 ±.14	20.4 ±.03	37.6 ±.07	20.8 ±.03		
Standard de- viation	121 ±2.1	1.6 ±.03	1.4 ±.02	3.5 ±.06	5.6 ±.10	1.4 ±.02	2.4 ±.05	1.2 ±.02		

Probable errors: < .06

*Sires' and average daughters' conformation*

Daughters' weight = 582 + .25 sires' weight  
 Daughters' height at withers = 34.9 + .25 sires' height at withers  
 Daughters' depth at withers = 20.0 + .24 sires' depth at withers  
 Daughters' heart girth = 48.4 + .28 sires' heart girth  
 Daughters' paunch girth = 63.0 + .26 sires' paunch girth  
 Daughters' width at hips = 9.1 + .54 sires' width at hips  
 Daughters' body length = 23.9 + .34 sires' body length  
 Daughters' rump length = 11.0 + .44 sires' rump length

The correlations on the left to right diagonal line of table 1 are for the measurements alike in sire and daughter. These correlations are in general larger than those where the measurement taken on the sire is different from the measurement taken on the daughter. Such variations result from differential development and from the action of unlike factors in inheritance which affect the body parts separately. Other analytical material is necessary to bring this point into sharp detail.

The block of nine correlations at the extreme lower-right hand corner for the interrelations of width at hips, body length and rump length are distinctly larger than those for the other measurements. This fact will be noted in all of the following tables.

The size of the correlations is also important. Considering like measurements of sire and daughter, the first five measurements have correlations which are somewhat lower than those expected on random assortment of the Mendelian factors on which size may depend. (For a more extended discussion of this question with regard to milk yield and butter fat, see papers cited in reference 2 of the Literature Cited.) The other three are large enough or in one case too large. The quantitative facts, while pointing strongly to the inheritance of these characters along customary multiple factor Mendelian lines, need further amplifications by the data which follow.

#### INFLUENCE OF DAM'S CONFORMATION ON THAT OF HER DAUGHTERS

The data for the relation between the type of dams and the type of their daughters are more extensive than those of the sires and their daughters, there being practically twice as many daughter and dam pairs. There are 1549 measured daughters which come from measured dams. Furthermore at least half of these daughter-dam pair measurements include different dams, whereas not more than 125 different sires were actually included in the measurements, most of the 736 measurements coming from sires which had more than one daughter. The data on the relation between the dam and daughter are therefore more reliable in this sense than those between sire and daughter. They are more obviously influenced by environment also as the dam and daughter are frequently raised in the same dairy barn. The mean types of the dams and daughters are shown in table 3.

The measurements of table 4 support the conclusion that the dam's conformation predicts the daughter's probable form. The results, however, are fairly irregular, indicating that the relation is not a close one. The data of table 3 are presented in graphical form in figure 3.

The irregular line in figure 3 shows the actual average daughter's size for a given conformation of the dam. The heavy line shows those points based on more than 10 daughters in determining the average. The straight line



TABLE 3  
*Relation between dam's type and that of her daughter.*

WEIGHT		HEIGHT AT WITHERS		DEPTH AT WITHERS		HEART GIRTH		PAUNCH GIRTH		WIDTH AT HIPS		BODY LENGTH		RUMP LENGTH	
DAMS'	DAUGHTERS'	DAMS'	DAUGHTERS'	DAMS'	DAUGHTERS'	DAMS'	DAUGHTERS'	DAMS'	DAUGHTERS'	DAMS'	DAUGHTERS'	DAMS'	DAUGHTERS'	DAMS'	DAUGHTERS'
AVERAGE	AVERAGE	AVERAGE	AVERAGE	AVERAGE	AVERAGE	AVERAGE	AVERAGE	AVERAGE	AVERAGE	AVERAGE	AVERAGE	AVERAGE	AVERAGE	AVERAGE	AVERAGE
425	1125	42.5	46.3	20.5	27.5	61	67.3	67	91.0	16.5	18.2	29.5	38.5	16.5	20.5
575	975	43.5	46.2	21.5	27.5	63	68.4	69		17.5	18.8	30.5		17.5	18.8
625	865	44.5	46.5	22.5	26.0	65	67.0	71	79.6	18.5	19.1	31.5	36.0	18.5	18.9
675	920	45.5	46.9	23.5	26.2	67	68.7	73	83.0	19.5	19.6	32.5	34.9	19.5	19.8
725	806	46.5	47.1	24.5	26.7	69	69.7	75	98.5	20.5	20.1	33.5	35.1	20.5	20.4
775	835	47.5	47.5	25.5	26.7	71	70.7	77	82.2	21.5	21.1	34.5	35.3	21.5	21.3
825	875	48.5	48.1	26.5	27.2	73	69.8	79	83.8	22.5	21.6	35.5	36.3	22.5	21.7
875	885	49.5	48.4	27.5	27.0	75	70.4	81	84.6	23.5	21.8	36.5	36.6	23.5	22.4
925	900	50.5	48.9	28.5	27.9	77	72.8	83	85.4	24.5	21.9	37.5	37.2	24.5	22.1
975	932	51.5	45.6	29.5	28.0	79	74.0	85	85.8	25.5		38.5	38.1		
1025	963	52.5	48.8	30.5	28.4	81	74.7	87	86.8	26.5		39.5	38.6		
1075	975			31.5	28.3	83	75.0	89	88.0	27.5	17.5	40.5	38.6		
1125	995			32.5	29.0			91	89.2	28.5	18.5	41.5	39.7		
1175	1010							93	88.6			42.5	40.3		
1225	1005							95	89.4			43.5	38.3		
1275	1025							97	89.8			44.5	39.5		
								99	90.6			45.5	37.5		
								101	94.1			46.5	38.0		
								103							

TABLE 4  
*Relation between the conformation of the daughter and that of her dam.*

DAMS' MEASUREMENTS	DAUGHTERS' MEASUREMENTS									
	WEIGHT	HEIGHT AT WITHERS	DEPTH AT WITHERS	HEART GIRTH	PAUNCH GIRTH	WIDTH AT HIPS	BODY LENGTH	RUMP LENGTH	DAMS' AVERAGE SIZE	STANDARD DEVIATION
Weight	.37	.32	.26	.26	.24	.15	.18	.20	917 ± 1.9	113 ± 1.4
Height at withers	.25	.40	.27	.25	.16	.19	.19	.25	47.6 ± .03	1.7 ± .02
Depth at withers	.21	.31	.32	.35	.27	.34	.30	.38	27.5 ± .02	1.5 ± .02
Heart girth	.18	.27	.32	.42	.34	.43	.34	.44	71.1 ± .06	3.5 ± .04
Paunch girth	.15	.23	.28	.37	.35	.36	.33	.38	87.5 ± .10	5.4 ± .06
Width at hips	.10	.21	.26	.40	.33	.59	.50	.58	20.3 ± .02	1.6 ± .02
Body length	.16	.29	.33	.36	.29	.49	.49	.46	37.1 ± .05	2.4 ± .03
Rump length	.11	.25	.31	.43	.34	.58	.49	.64	20.5 ± .02	1.4 ± .02
Daughters' average size	905 ± 2.1	47.6 ± .03	27.5 ± .02	70.7 ± .06	87.0 ± .10	20.2 ± .02	37.1 ± .05	20.5 ± .02		
Standard deviation	120 ± 1.5	1.6 ± .02	1.4 ± .02	3.7 ± .04	5.7 ± .07	1.5 ± .02	2.3 ± .03	1.3 ± .02		

Probable errors 0.02 when  $r$  less than 0.3, otherwise the probable errors are 0.01.

Daughters' weight = 540 + .398 Dams' weight  
 Daughters' height at withers = 28.7 + .398 Dams' height at withers  
 Daughters' depth at withers = 18.9 + .312 Dams' depth at withers  
 Daughters' heart girth = 38.7 + .450 Dams' heart girth  
 Daughters' paunch girth = 54.4 + .373 Dams' paunch girth  
 Daughters' width at hips = 8.9 + .555 Dams' width at hips  
 Daughters' body length = 19.3 + .481 Dams' body length  
 Daughters' rump length = 8.0 + .612 Dams' rump length

indicates the average weighted trend of the data as determined from the proper equations indicated in table 4.

The size of the daughters of a small Jersey cow compared with the size of

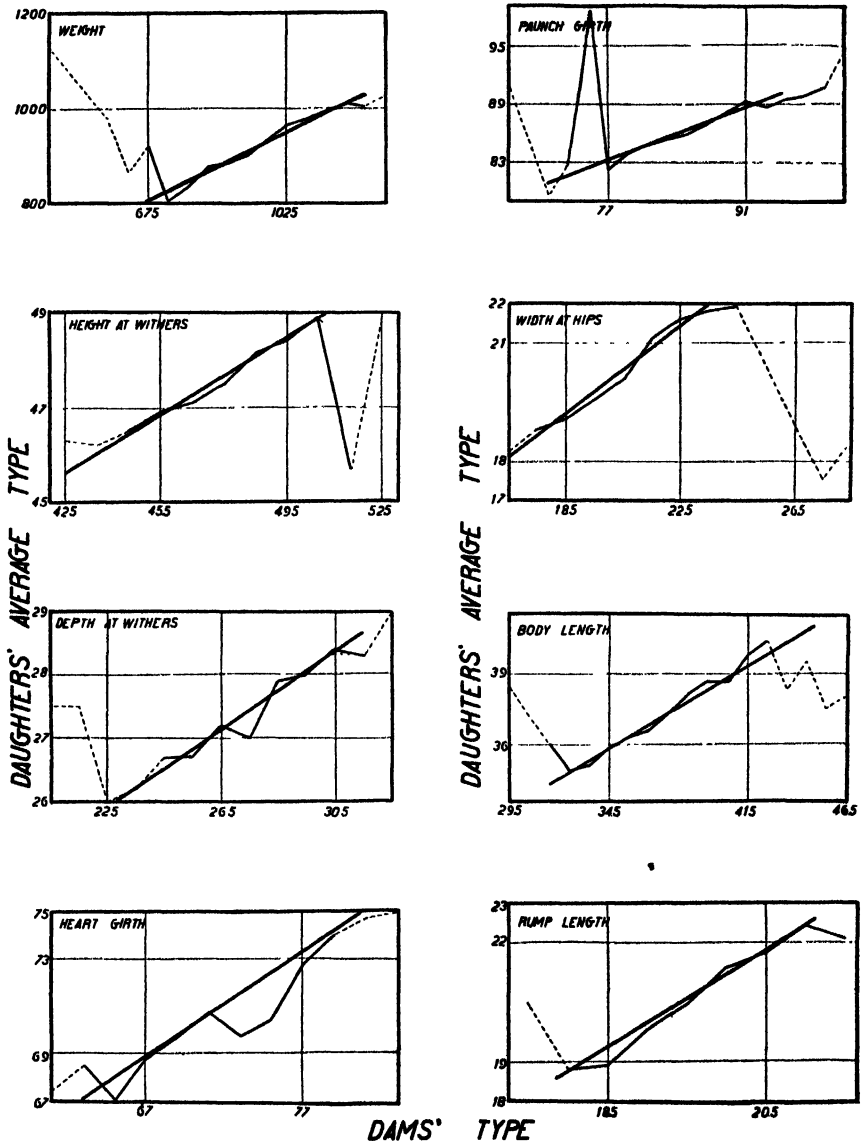


FIGURE 3.—The relation between the conformation of the dam and the average conformation of her daughter.

the daughters of a large Jersey cow indicates what size may mean to the offspring when considered on the basis of the extremes of the race. If the dam is 575 pounds, the average size of the daughter is 769 pounds. If the

dam is 1225 pounds, the average size of the daughter is 1028 pounds. An increase in size of 650 pounds for the dam has resulted in an average increase in size for the daughters of 259 pounds. For height at withers dams of 41.5 inches have, on the average, daughters of 45.2 inches in size. Where the dam is 51.5 inches at the withers, the average daughter's size is 49.2 inches. It is clear that the daughters tend to approach the average although they still show the effect of the dams from which they come. An increase of 10 inches for the size of the dam at height at withers has resulted in an increase of 4 inches for the daughter. A similar condition is found for the other body measurements.

The correlation coefficients and the proper equations for relating the size of the dam to that of the daughter are given in table 4.

The data of table 4 are comparable with those of table 2 for the sire. The average correlation coefficient between the daughters and sires on like measurements is 0.39 and that for the dams and daughters is 0.45. These correlations are not significantly different although they indicate a slight influence of the more common environment of the dam and daughter. If we are to interpret these relationships as due to the inheritance of size on a multiple factor basis the numerical value is somewhat low. The interrelation of the unlike body parts shows somewhat the same tendency. The mean correlation coefficient for the sires is 0.27 and that for the dams is 0.30.

#### RELATION BETWEEN THE CONFORMATIONS OF FULL SISTERS

Full sisters are, from an inheritance standpoint, as closely related individuals as it is possible to have in only one generation of matings. It would be expected that full sisters would resemble each other closely if inheritance in any way played a part in determining the conformation of the animal. It would also be expected, in view of the fact that most full sisters are brought up under the same conditions, in the same barn, by the same owner, that environment too would be an important factor in bringing about a close resemblance between such relatives. The effect of environment would undoubtedly be much greater for such full sisters than for either a sire and his daughter or a dam and her daughter. The results of the comparison of full sisters are important in showing the combined influence of these two factors in molding the conformation of the animal to the desired type. The measurements on 292 full sisters, making a total of 802 pairs, have been compared for the eight items of conformation.

The results of this study show that the conformations of full sisters are more closely related in the different measurements than are either sire and daughter or dam and daughter. The correlation coefficients showing the degree of these relationships are given in table 5. The close relation which

TABLE 5  
*Relation between the conformation of one sister with that of her full sister.*

FULL SISTER'S MEASUREMENTS	SISTER'S MEASUREMENTS	CORRELATION COEFFICIENTS	MEAN	STANDARD DEVIATION
Weight	Weight	.49	936 $\pm$ 3.3	125 $\pm$ 2.3
Height at withers	Height at withers	.43	48.0 $\pm$ .05	1.66 $\pm$ .03
Depth at withers	Depth at withers	.33	27.7 $\pm$ .05	1.38 $\pm$ .03
Heart girth	Heart girth	.40	71.6 $\pm$ .10	3.57 $\pm$ .07
Paunch girth	Paunch girth	.37	88.2 $\pm$ .14	5.08 $\pm$ .10
Width at hips	Width at hips	.65	20.5 $\pm$ .04	1.44 $\pm$ .03
Body length	Body length	.54	37.6 $\pm$ .12	2.53 $\pm$ .08
Rump length	Rump length	.64	20.7 $\pm$ .04	1.29 $\pm$ .02

Full sister's weight	= 475.2 + .49 sister's weight
Full sister's height at withers	= 27.5 + .43 sister's height at withers
Full sister's depth at withers	= 18.5 + .33 sister's depth at withers
Full sister's heart girth	= 43.0 + .40 sister's heart girth
Full sister's paunch girth	= 55.2 + .37 sister's paunch girth
Full sister's width at hips	= 7.1 + .65 sister's width at hips
Full sister's body length	= 17.4 + .54 sister's body length
Full sister's rump length	= 7.4 + .64 sister's rump length

exists between these full sisters may be indicated in the following way. If the full sisters of cows weighing 800 pounds are compared with the full sisters of cows weighing 900 pounds, the difference in the average weight of these full sister groups would furnish an indication of the influence of differences of 100 pounds of weight on the weight of another full sister. By such a comparison, it is found that an increase in any of the type measurements results in an increase in the type measurements of the second full sisters as follows: A cow which weighs 100 pounds more than another cow tends to have full sisters 49 pounds heavier than those of the smaller cow. Similarly, for cows differing by one inch in size, the larger cow tends to have full sisters 0.42 inches larger in height at withers, 0.34 inches in depth at withers, 0.40 inches in heart girth, 0.37 inches in paunch girth, 0.65 inches in width at hips, 0.54 inches in body length, and 0.64 inches in rump length. It will be noted that the rates of increase are essentially the same for the four measurements, height at withers, depth at withers, heart girth, and paunch girth. Those for width at hips, body length, and rump length are considerably greater. The results are throughout concordant in showing the marked influence of the cow's type on that of her full sister.

The correlation coefficients measuring the relation between full sisters, together with the equations showing the mean values for the measurements, are found in table 5. The average correlation is 0.48, a correlation which is quite similar to that expected for full sisters on random mating and complete determination by heredity. In view of the divergence of the first five correlations from the values of the last three, this relation has little meaning without the other data which are to follow.

RELATION BETWEEN THE TYPE OF ONE HALF SISTER WITH THAT OF  
ANOTHER WHEN THE SIRE IS THE COMMON PARENT

Half sisters may have a common sire or a common dam. On an inheritance basis they should have approximately only half the inheritance in common which is found for full sisters.

On the other hand, half sisters have the same opportunity with full sisters to be affected by the different feeding, management and selection practices of the various farms from which they come. Most half sister groups come from the same farms rather than different farms and would therefore tend to be alike both because of their inheritance and their environment. Taking these factors into account, the degree of resemblance between full sisters on the one hand would be in its simplest form:

$$1/2 \text{ heredity}^2 + \text{environment}^2$$

while for half sisters:

$$1/4 \text{ heredity}^2 + \text{environment}^2$$

The expectation would consequently be that the half sisters would resemble each other less than full sisters in their size measurements; the differences being interpreted as the effects of the environment. If selection and care, et cetera, have no effect, then the half sisters should resemble each other only half as much as the full sisters. If environment plays an important role, the half sisters will be nearly as much alike as the full sisters. If heredity has no effect the half sisters will be as much alike as full sisters. The full sister groups and half sister groups, coming as they do from all manner of farms each of which have their chance to influence the conformation of pairs of sisters, give the environmental factors full play and push to the background the influence of heredity. Any explanation of the results must take cognizance of the influence of the two components of heredity and environment so that the facts may be truly comparable with those previously obtained in the parent and offspring groups.

If the cow's conformation be contrasted with that of her half sister, on the same basis as that used for the full sisters, it is found that for an increase of 100 pounds in weight the larger cow tends to have half sisters 38 pounds heavier than the smaller cow. An increase of one inch in any of the body measurements results in the larger cow having half sisters larger by the following amounts: height at withers 0.34 inches, depth at withers 0.28 inches, heart girth 0.37 inches, paunch girth 0.31 inches, width at hips 0.67 inches, body length 0.60 inches, and rump length 0.50 inches. It is clear that the half sisters do not resemble each other to quite the same extent as full sisters do. It is also noted that the first five measurements tend to have the same relative effect, whereas the last three tend to have a much greater effect, so far as the influence of the size of one half sister

on that of the other is concerned. Some factor, not as yet clear, tends to make the cows resemble each other more closely in width at hips, body length, and rump length than they do for the other five measurements, weight, height at withers, depth at withers, heart girth, and paunch girth.

The correlations measuring these relationships are shown in table 6.

TABLE 6

*Relation between the conformation of one sister with that of her half sister on the sire's side.*

HALF SISTER'S MEASUREMENTS	SISTER'S MEASUREMENTS	CORRELATION COEFFICIENTS	MEAN	STANDARD DEVIATION
Weight	Weight	.38	920 ± 1.3	116 ± .9
Height at withers	Height at withers	.34	47.7 ± .02	1.63 ± .01
Depth at withers	Depth at withers	.28	27.6 ± .02	1.45 ± .01
Heart girth	Heart girth	.37	71.4 ± .04	3.71 ± .03
Paunch girth	Paunch girth	.31	87.8 ± .06	5.48 ± .04
Width at hips	Width at hips	.67	20.6 ± .02	1.51 ± .01
Body length	Body length	.50	37.7 ± .03	2.44 ± .02
Rump length	Rump length	.60	20.9 ± .02	1.38 ± .01

Half sister's weight = 574 + .38 sister's weight  
 Half sister's height at withers = 31.5 + .34 sister's height at withers  
 Half sister's depth at withers = 19.8 + .28 sister's depth at withers  
 Half sister's heart girth = 44.8 + .37 sister's heart girth  
 Half sister's paunch girth = 60.7 + .31 sister's paunch girth  
 Half sister's width at hips = 6.8 + .67 sister's width at hips  
 Half sister's body length = 18.8 + .50 sister's body length  
 Half sister's rump length = 8.4 + .60 sister's rump length

#### RELATION BETWEEN THE CONFORMATION OF ONE HALF SISTER WITH THAT OF ANOTHER WHEN THE DAM IS THE COMMON PARENT

Half sisters with a common dam have the same common inheritance as half sisters with a common sire.

If the influence of one half sister upon another be determined in the manner previously used, it is found that a cow which is 100 pounds heavier than another will tend to have half sisters 39 pounds heavier than those of the smaller cow. Similarly the cow which is larger by one inch for the other body measurements will have sisters larger by the following amounts: 0.32 for height at withers; 0.26 for depth at withers; 0.42 for heart girth; 0.38 for paunch girth; 0.55 for width at hips; 0.48 for body length; and 0.60 for rump length. These results show clearly that the size of the cow materially affects the size of her half sisters. This result is again most pronounced for width at hips, body length, and rump length, but is significant for weight, height at withers, depth at withers, paunch girth, and heart girth.

The correlation coefficients of table 7 for the interrelation of these half sisters' measurements have a mean value of 0.42. This value is identical with that of the half sisters on the sire's side and somewhat less than that for the full sisters.

TABLE 7

*Relation between the conformation of one sister with that of her half sister on the dam's side.*

HALF SISTER'S MEASUREMENTS	SISTER'S MEASUREMENTS	CORRELATION COEFFICIENTS	MEAN	STANDARD DEVIATION
Weight	Weight	.39	917 ± 2.7	117 ± 1.9
Height at withers	Height at withers	.32	47.7 ± .04	1.66 ± .03
Depth at withers	Depth at withers	.26	27.5 ± .03	1.44 ± .02
Hearth girth	Heart girth	.42	71.1 ± .08	3.53 ± .06
Paunch girth	Paunch girth	.38	87.5 ± .13	5.66 ± .09
Width at hips	Width at hips	.55	20.3 ± .03	1.46 ± .02
Body length	Body length	.48	37.1 ± .06	2.33 ± .04
Rump length	Rump length	.60	20.5 ± .03	1.29 ± .02

Half sister's weight = 557 + .39 sister's weight  
 Half sister's height at withers = 32.2 + .32 sister's height at withers  
 Half sister's depth at withers = 20.3 + .26 sister's depth at withers  
 Half sister's heart girth = 41.5 + .42 sister's heart girth  
 Half sister's paunch girth = 54.3 + .38 sister's paunch girth  
 Half sister's width at hips = 9.2 + .55 sister's width at hips  
 Half sister's body length = 19.3 + .48 sister's body length  
 Half sister's rump length = 8.2 + .60 sister's rump length

#### ASSORTIVE MATING IN JERSEY CATTLE

By assortive mating is meant the tendency of breeders to mate individuals which are alike. This similarity may be taken as a whole or it may be for some given part of the body. Assortive mating is thus a distinct and fundamental function in any breeding, whether the procedure be deliberately controlled or not.

In regard to one character, milk yield, it was found that assortive mating did not occur to the extent which might be expected. This was no doubt due in large part to the fact that milk yield is a relatively obscure character requiring a large volume of records for its appreciation and control, a fact tending to reduce the number of those willing to give it sufficient attention to make for an effective assortive mating coefficient. The case for the body characteristics of the dairy cow is not of this kind. The relative size of any of the characters may be readily appreciated by simply looking at the cow. Dairy-men are continually making comparisons of this kind and naturally become expert in taking into account such things as relation of size to age, thus making the selection more effective.

Assortive mating coefficients thus have an interest to all breeders because they measure the effective selection and breeding which is taking



place within the breed for the given character. They have a further interest in that the degree to which the parents are selected for a given character markedly influences the amount of resemblance which will be found between the parents and their offspring.

If assortive mating is to be a real force in molding the characteristics of a breed it must do several things. The first and most important is that the sires to be used in the different herds must, in large part, come from superior parents. The sires selected for actual breeding must themselves be the best of the progeny of such parents. Sires of this calibre should then have the best mates which the breed affords. Some appreciation of how active is this selective breeding may be had by a comparison of the relative sizes of the sires and the cows to which they are bred. The results of this comparison for the eight body measurements of conformation are shown in the averages which follow; the sires being divided into groups, from the smallest to the largest, three groups in all and their mates' average sizes determined.

WEIGHT		HEIGHT AT WITHERS		DEPTH AT WITHERS		HEART GIRTH	
SIRE	MATE	SIRE	MATE	SIRE	MATE	SIRE	MATE
1199	880	49.9	47.4	29.7	27.3	74.3	70.0
1430	925	52.0	48.0	32.1	27.5	80.0	71.0
1570	975	54.5	47.6	34.8	27.5	84.2	72.0

PAUNCH GIRTH		WIDTH AT HIPS		BODY LENGTH		RUMP LENGTH	
SIRE	MATE	SIRE	MATE	SIRE	MATE	SIRE	MATE
84.0	85.6	19.1	19.5	37.1	36.2	21.2	19.9
92.0	88.6	20.8	20.2	41.1	36.8	22.5	20.6
99.1	88.0	23.0	22.0	43.5	38.5	24.3	21.8

It will be recalled that all measurements presented are mature form measurements. The data cited above were obtained in the following manner. There were 103 matings of sires and dams in which the sire's weight was less than 1350 pounds. These sires' weights averaged 1199 pounds. They were bred to cows whose average weight was 880 pounds. There were 167 matings in which the sire's weight ranged between 1350 and 1500 pounds with an average of 1430 pounds. They were bred to cows averaging 925 pounds. Seventy-seven matings had sires whose weights were above 1500 pounds. These sires averaged 1570 pounds in weight and were bred to cows averaging 975 pounds in weight. There is a distinct increase in the weight of the sires' mates as the weight of the sires increases. This correlation is carried through the other data particularly for the width at hips, body length and rump length. These results may be seen graphically in figure 4.

The degree of selection practiced in the Jerseys is evidently pronounced for some of the regions of the body conformation, less in others and almost non-existent in the remainder. The regions chosen for emphasis in breeding are width at the hips, body length and rump length. Less effective selection

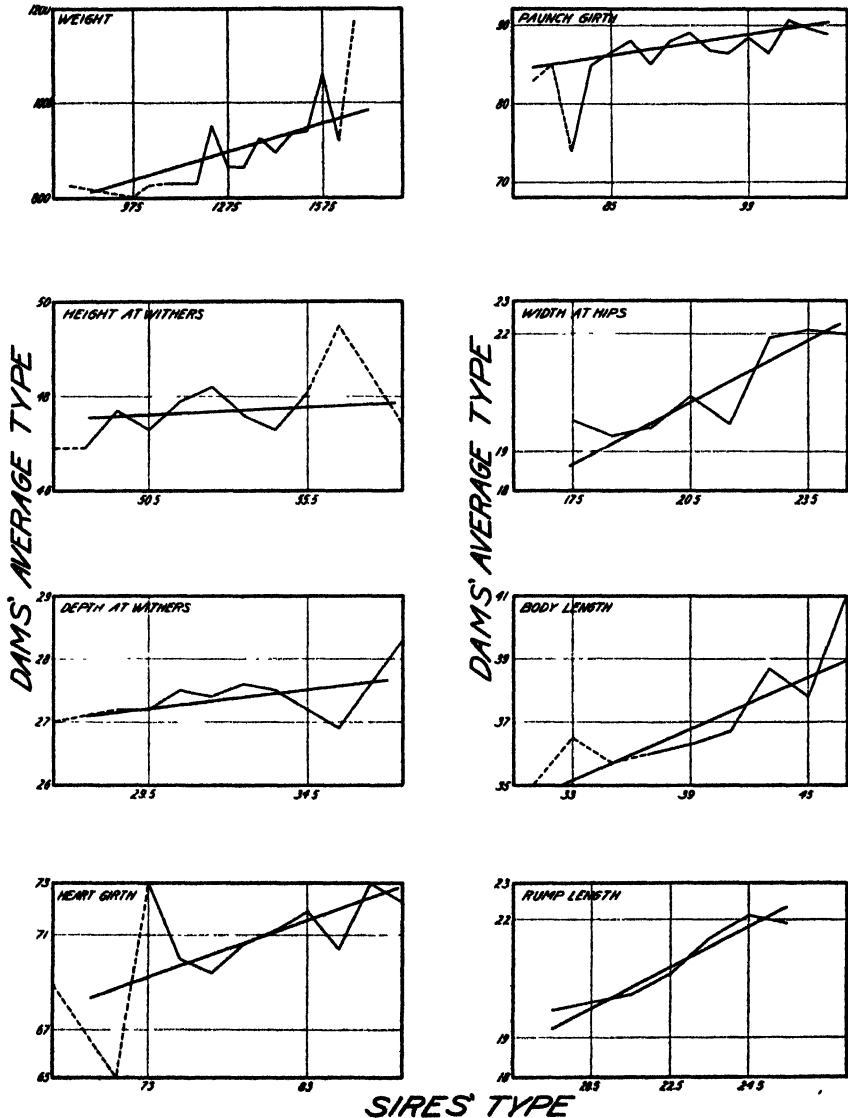


FIGURE 4.—Relation between the size of the sire and the size of his mates for Jersey cattle.

is seen in body weight, heart girth and paunch girth. Little selection is practiced for height at withers and depth at withers. Herd owners very evidently believe that if they have cows which have wide hips, long bodies or rumps, that these cows should be bred to bulls having the same qualities,

whereas if their cows have narrow hips, short bodies or rumps, they should be bred to bulls which, relatively speaking, have equally narrow hips, short bodies and rumps. A like, though less extreme, belief also appears to exist with regard to the weight, heart girth and paunch girth. The matings with regard to the height at withers and depth at withers appear to be almost random matings, a sire high at the withers being proportionately as often bred to cows which were low at the withers, high at the withers, or medium in height.

Two factors appear to be capable of giving the degree of assortive mating observed. The first is that breeders may believe that the conformational characteristics, width at hips, rump length and body length, and, to a lesser degree, weight, paunch girth and heart girth in the order named are factors of importance in indicating production. If such a belief is sufficiently strong to cause these points to have a financial premium the desired sires and dams would be concentrated into the hands of the wealthy, the less desired stock would be in the herds of breeders with less wealth, thus giving the observed results. This situation could be accentuated by a second factor working in conjunction with it. This second factor is the tendency, perhaps stronger in the Jersey breed than in most others, of confining the breeding closely within family lines. If these family lines differ by characteristics such as width at hips, body length, et cetera, these characteristics being inherited and selected according to the particular whim of the breeders establishing the family, they would tend to make diverse lines for these characteristics. A population composed of such distinct families would give the assortive mating coefficients like those observed. In passing it is of point to suggest that it is unfortunate that the points of conformation most important to production were not emphasized in this selection: weight and heart girth.

The relative intensity of the selective or assortive breeding going on within the herds of Jerseys in this country is best appreciated by an examination of the correlation coefficients between the measurements of sires and their mates. These correlation coefficients are given in the tables which follow.

TABLE 8  
*Correlation coefficients between the sire's measurements and those of his mates.*

CORRELATION COEFFICIENTS	
Weight	0.26 ± .03
Height at Withers	.08 ± .04
Depth at Withers	.07 ± .04
Hearth Girth	.18 ± .03
Paunch Girth	.22 ± .03
Width at Hips	.65 ± .02
Body Length	.40 ± .03
Rump Length	.52 ± .03

The first five assortive mating coefficients of table 8 correspond well with those which PEARSON (3) has calculated for stature, span and forearm length in man. The last three coefficients differ from either set in being much larger. The size of these coefficients suggests that they are in part responsible for the observed difference in the ancestral correlations of these same groups. The analysis of this point is presented in the following section.

#### RELATIVE INFLUENCE OF HEREDITY AND ENVIRONMENT ON CONFORMATION WITHIN THESE JERSEYS

The somatic appearance of an animal is due, first, to the genetic constitution of the fertilized egg and, second, to the environmental opportunity which is afforded the egg for development up to adult size. Assortive mating based on somatic appearance of the animal would consequently be attributed to both heredity and environment.

The definite tendency of Jersey breeders to breed within families would give the assortive mating coefficients a different emphasis. The families within the Jerseys are genetically different in origin. The average environment as represented by care, climate, feeding, et cetera, is throughout essentially the same between the different families. There are good, bad and indifferent cattlemen growing cattle in each family. The magnitude of the assortive mating coefficient when the breeding is carried on within such sub-groups is consequently dependent on their genetic background.

These two interpretations appear to be the major possibilities in analyzing the coefficients of assortive mating. There is another possibility, however; namely, that in taking the measurements the standard of measurement was different between the men making the measurements. This possibility can be eliminated since the correlations for the data of the two men derived separately lead to essentially the same numerical results. To remove any possible objection on this ground we shall consider only the data taken by one man, measuring the larger series of about 5000 animals.

As WRIGHT, FISHER and others (4, 5) have pointed out, the parent and offspring correlations are less than the full sister correlations by an amount equal to the effect of the common environment or, as FISHER (6) has shown to the influence of dominance. In our case any difference between the sire and daughter correlations and the dam and daughter correlations might be considered due to the more common environment of dam and daughter in their development. In his systems of mating, WRIGHT<sup>2</sup> has analyzed these assumptions and derived general formulae for them. His symbols are  $h^2$  determination of size by heredity,  $d^2$  determination of heredity by dominance deviations,  $e^2$  determination of size by the environment com-

<sup>2</sup> The writer is indebted to Dr. WRIGHT for much in the discussion which follows.

mon to full or half sisters,  $m$  the correlation between genetic constitution of mates, and  $m_1$  correlation between genotypes of different mates. The effects of the common environment and of dominance could theoretically be combined in all gradations. Such gradation would offer serious complication in the analysis of the problem. An estimate of the effect of common environment and dominance can be reached, however, (1) by assuming the dominance effect to be non-existent, and (2) by assuming the environmental effect to be absent and the dominance to account for the differences. By contrasting the two results it is then possible to see what effect each of the variables really has. The equations which follow on the various assumptions are listed below:

(1) Assortive mating is somatic and the genetic factors are without dominance.

$$r_{sd} = \frac{m}{h^2} \quad r_{so} = r_{do} = 1/2h^2(1 + r_{sd})$$

$$r_{oo} = 1/2h^2(1 + m) + e^2 \quad r_{oo}' = 1/4h^2(1 + 2m + m^1) + e_1^2$$

Where  $s$  = sire,  $d$  dam,  $o$  daughter,  $oo$  full sister and  $oo^1$  half sister.

(2) Assortive mating as above, but difference between dam and daughter correlations and sire and daughter correlations assumed due to the more common environment of dam and daughter as contrasted with that of sire and daughter. The only change which this assumption makes in the above equations is

$$r_{sd} = 1/2h^2(1 + r_{sd}) \text{ as before but } r_{do} = 1/2h^2(1 + r_{sd}) + e^2$$

(3) Assortive mating as above but with genetic factors showing dominance and no effect of environment.

$$r_{sd} = \frac{m}{h^2(1 - d^2)} \quad r_{so} = r_{do} = 1/2h^2(1 - d^2)(1 + r_{sd})$$

$$r_{oo} = 1/2h^2(1 - \frac{d^2}{2})(1 + m) \quad r_{oo}' = 1/4h^2(1 - d^2)(1 + 2m + m^1)$$

(4) Assortive mating is genetic and no dominance of factors, environment as before.

$$r_{sd} = h^2m \quad r_{so} = r_{do} = 1/2h^2(1 + m)$$

$$r_{oo} = 1/2h^2(1 + m) + e_1^2 \quad r_{oo}' = 1/4h^2(1 + 2m + m^1) + e_1^2$$

The correlation coefficients which are derived from the series of measurements taken by the man measuring the largest series are shown below. These correlations will be noted to be entirely similar to those derived from the combined data of both men. There was consequently no divergence of the standard used by the two men in measuring their animals.

TABLE 9

	$r_{eo}$	$r_{do}$	$r_{ed}$	$r_{oo}$	$r_{oo}'$ sire	$r_{oo}'$ dam
Weight	0.349	0.382	0.212	0.493	0.353	0.291
Height at Withers	0.267	0.336	0.013	0.383	0.306	0.223
Depth at Withers	0.359	0.312	0.108	0.304	0.283	0.219
Heart Girth	0.350	0.436	0.218	0.450	0.381	0.416
Paunch Girth	0.299	0.376	0.204	0.383	0.320	0.377
Width at Hips	0.686	0.622	0.607	0.688	0.614	0.579
Body Length	0.407	0.500	0.326	0.492	0.505	0.513
Rump Length	0.569	0.679	0.571	0.705	0.615	0.629

The solution of the various equations under the above assumptions is shown below.

The data of table 10 enable us to form some judgment regarding the influence of the various factors which affect size inheritance. The hypotheses considered are the major simple ones. Theoretically they could be combined in a variety of ways. The results from such combinations would be intermediate, however, so that conclusions arrived at on the basis of this material would be essentially correct for such intermediate hypotheses.

The data under hypotheses (1) and (2) with assortive mating somatic and no dominance of the genetic factors for size and that for (4) with assortive mating genetic and no dominance of these same factors, give the factor environment all the possible credit it can have in determining the constitution of these Jersey cattle. The data show that the environmental effects are negligible. In fact it will be noted that several of the constants take negative values. The facts in these dairy cattle consequently agree with those of PEARSON and LEE (3) on human constitution in showing but little effect on the constitution of the environmental variation which differentially played upon the individuals within the populations studied.

The hypothecating of dominance in the factors for size as seen in (3) leads to high values for the effect of heredity; three values being in fact indeterminate. The values for the correlation between the genotypes of mates are also high, four out of the eight being indeterminate. These facts seem to give suitable grounds for discarding the dominance hypothesis for the size factors in these dairy cattle.

Further information by which to differentiate between the effectiveness of the other hypotheses is furnished by the genetic correlations for the ancestral combinations as calculated from these data.

The consequences following from these three hypotheses as seen in tables 10 and 11 certainly suggest that (4), assortive mating of genetic origin and no dominance is not applicable to these Jersey cattle. The genetic correlations are throughout too high. Such correlations would require the Jerseys to be split into families which were only bred within each other and never

TABLE 10  
*Relative influence of heredity  $h^2$ , environment  $e^2$ , dominance  $d^2$ , and the correlation of genotypes  $m, m^1$*

	(1)				(2)				(3)				(4)			
	$h^2$	$e^2$	$m$	$m^1$	$h^2$	$e^2$	$m$	$m^1$	$h^2$	$d^2$	$m$	$m^1$	$h^2$	$e^2$	$m$	$m^1$
Weight	0.60	0.15	0.13	-0.14	0.58	0.17	0.03	0.12	1.22	0.50	0.13	0.88	0.52	0.13	0.41	0.32
Height at Withers	0.60	0.06	0.08	0.21	0.53	0.10	0.07	0.07	0.85	0.30	0.08	0.62	0.59	0.08	0.02	0.20
Depth at Withers	0.61	-0.02	0.07	0.41	0.65	-0.04	-0.05	0.07	0.68	0.12	0.07	0.53	0.56	-0.03	0.19	0.18
Heart Girth	0.65	0.08	0.14	0.68	0.58	0.13	0.09	0.13	0.98	0.34	0.14	1.19	0.57	0.06	0.38	0.63
Panneh Girth	0.56	0.07	0.11	0.75	0.50	0.11	0.08	0.10	0.85	0.34	0.11	1.26	0.47	0.05	0.43	0.70
Width at Hips	0.81	0.08	0.50	0.55	0.85	0.04	-0.06	0.52	1.13	0.28	0.50	0.94	0.70	0.03	0.87	0.48
Body Length	0.68	0.07	0.22	1.10	0.61	0.12	0.09	0.20	1.11	0.98	0.30	1.53	0.58	0.04	0.56	1.12
Rump Length	0.79	0.13	0.45	0.59	0.72	0.19	0.11	0.41	1.30	0.39	0.45	1.23	0.68	0.08	0.84	0.51
Average	0.66	0.08	0.21		0.63	0.11		0.20		0.32	0.21		0.58		0.46	
	$\pm .02$	$\pm .01$	$\pm .04$		$\pm .03$	$\pm .01$		$\pm .04$		$\pm .02$	$\pm .04$		$\pm .02$		$\pm .06$	

outside. This type of breeding is partly true but inbreeding should be widespread if it were as active as the correlations under (4) would necessitate.

TABLE 11  
*Genetic correlations.*

	(1)			(2)			(4)		
	$r_{po}$	$r_{oo}$	$r_{oo'}$	$r_{po}$	$r_{oo}$	$r_{oo'}$	$r_{po}$	$r_{oo}$	$r_{oo'}$
Weight	0.61	0.56	0.28	0.61	0.56	0.26	0.70	0.70	0.53
Height at Withers	0.51	0.54	0.34	0.51	0.53	0.31	0.51	0.51	0.31
Depth at Withers	0.55	0.53	0.39	0.55	0.54	0.45	0.60	0.60	0.39
Heart Girth	0.61	0.57	0.49	0.61	0.56	0.47	0.69	0.69	0.60
Paunch Girth	0.60	0.56	0.50	0.60	0.55	0.48	0.72	0.72	0.64
Width at Hips	0.80	0.75	0.64	0.80	0.76	0.65	0.93	0.93	0.80
Body Length	0.66	0.61	0.64	0.66	0.66	0.63	0.78	0.78	0.81
Rump Length	0.79	0.73	0.62	0.79	0.71	0.59	0.92	0.92	0.80

Hypotheses 1 and 2 are essentially the same in view of the lack of any environmental effect. The results are, considering their probable errors, reasonable although the variation covered is quite wide. The greatest complicating factor is the half sister correlations which are too large. The best hypothesis would seem to be that part of the assortive mating is somatic and part genetic, the factors for size being without dominance and mating taking place in such a manner as to form partially non-interbreeding families.

That variation in heredity is the main cause of the variation in the size of Jersey cattle is evident from this analysis. The close relation between the genotypes of the mates has resulted from the type of breeding. It is of interest to note how far this inbreeding and selection has been carried by the herd owners within their individual herds and how much residual variation

TABLE 12  
*Correlation coefficients for the relation of dam and daughter type within certain herds.*

HERD NO.	WEIGHT	HEIGHT AT WITHERS	DEPTH AT WITHERS	HEART GIRTH	PAUNCH GIRTH	WIDTH AT HIPS	BODY LENGTH	RUMP LENGTH	NO. OF PAIRS
19	-0.22	-0.13	-0.04	-0.01	0.03	-0.01	0.15	0.02	27
27	-0.03	0.10	-0.32	-0.11	-0.20	0.08	0.31	0.20	25
47	0.06	-0.38	0.26	0.09	-0.31	0.08	0.17	0.10	23
52	0.45	0.34	0.27	0.43	0.28	0.32	0.16	0.39	20
58	0.53	0.49	0.65	0.32	0.49	0.34	0.18	0.60	27
69	0.30	0.26	0.18	0.17	0.35	0.51	0.35	0.16	27
77	-0.15	0.23	-0.13	-0.12	-0.02	-0.15	-0.15	0.15	50
78	0.06	0.25	0.01	-0.02	-0.02	-0.02	-0.12	0.04	63
88	0.33	0.34	0.44	0.30	0.31	0.15	0.15	0.21	27
102	0.03	0.20	0.18	0.05	-0.04	0.04	0.26	0.13	39
203	-0.02	0.39	0.20	0.12	-0.05	-0.08	0.07	0.34	31
Mean	0.12	0.19	0.15	0.11	0.07	0.11	0.13	0.20	



still exists which the individual breeder may utilize in further change of the constitution of his cows. This information may be derived from the correlations of conformations of cows within the individual herds. Because of the reduced number of animals available within individual herds only the correlations between daughter and dam, half sisters with a common sire and half sisters with a common dam have any meaning. These correlations are tabulated below for those herds which have 20 or more such pairs.

Table 12 shows that there is a fairly wide variation between the different herds in the amount of residual variation which exists within them. Some herds could change the form of their cattle by further selective breeding. The average residual heredity which remains out of the total which could have existed for the particular herd is rather small. This fact is further brought out in tables 13 and 14 for the two classes of half sisters.

TABLE 13  
*Correlation coefficients for the relation between the types of half sisters with a common sire.*

HERD NO.	WEIGHT	HEIGHT AT WITHERS	DEPTH AT WITHERS	HEART GIRTH	PAUNCH GIRTH	WIDTH AT HIPS	BODY LENGTH	RUMP LENGTH	NO. OF PAIRS
19	0.02	0.00	-0.04	0.00	-0.03	0.02	0.01	0.09	960
27	0.13	-0.02	0.05	-0.03	-0.03	-0.07	-0.04	-0.01	418
47	0.01	-0.05	0.03	-0.06	-0.05	-0.03	-0.06	-0.04	514
52	-0.04	0.11	-0.02	-0.05	-0.05	0.01	0.07	-0.01	346
58	-0.08	-0.10	0.06	-0.03	-0.05	0.13	0.00	0.00	850
69	0.08	-0.01	-0.02	0.09	0.05	0.04	-0.04	0.09	486
77	0.00	0.18	0.02	0.01	0.06	0.10	0.14	0.01	3644
78	0.10	0.07	0.16	0.21	0.15	0.33	0.15	0.19	3610
88	-0.02	0.06	-0.06	0.00	0.03	0.11	0.24	0.03	768
102	0.09	0.06	0.12	0.11	0.03	0.09	0.08	0.29	1462
203	-0.03	-0.01	-0.01	-0.23	-0.05	0.14	-0.05	-0.03	484
Mean	0.02	0.02	0.02	0.00	0.01	0.08	0.05	0.06	

TABLE 14  
*Correlation coefficients for the relation between the types of half sisters with a common dam.*

HERD NO.	WEIGHT	HEIGHT AT WITHERS	DEPTH AT WITHERS	HEART GIRTH	PAUNCH GIRTH	WIDTH AT HIPS	BODY LENGTH	RUMP LENGTH	NO. OF PAIRS
19	-0.23	0.13	-0.21	-0.33	-0.26	-0.42	0.05	-0.33	50
27	-0.13	0.38	0.02	0.47	-0.08	0.17	-0.42	-0.07	22
47	-0.22	-0.49	-0.07	-0.60	-0.17	-0.33	-0.20	-0.45	22
52	0.22	-0.36	-0.11	0.00	-0.35	-0.33	-0.17	-0.13	20
58	0.03	-0.25	-0.34	0.40	-0.20	0.15	-0.13	0.42	38
69	0.13	0.02	0.25	-0.06	0.23	-0.11	0.08	0.36	36
77	0.25	0.37	-0.11	0.47	0.37	0.51	0.24	0.29	44
78	0.31	0.19	0.18	0.19	0.09	0.24	0.10	0.30	116
88	0.07	-0.23	0.14	0.14	0.00	-0.09	-0.21	-0.33	34
102	-0.06	0.20	0.09	0.04	-0.01	-0.13	0.01	0.17	74
203	-0.39	-0.13	-0.57	-0.58	-0.55	-0.19	-0.03	-0.24	20
Mean	0.01	-0.02	0.06	0.01	-0.06	-0.05	-0.05	0.00	

The data of tables 12, 13 and 14 show that the Jersey herds have been differentiated into types which are quite pure in their inheritance. These types are different for different herds. The effect of the methods of breeding which have been employed have been to produce family groups which are rather homogeneous. To bring in genetic variability on which to work for further change would necessitate the interbreeding of some of these groups.

#### SUMMARY

This paper presents a summary of the body constitution of Jersey cattle as it is found after centuries of evolution under domestication and separation from other cattle for a period of nearly 200 years. The cattle utilized are pure-bred Jerseys owned within 15 states of the United States. Nearly 6000 relatively high-grade Jerseys form the basis for this study.

Data are presented to show the amount of residual inheritance for body constitution which remains within the breed as a whole and on the individual farms where the breeding is carried on. The parent and offspring and sibship correlations are presented. These correlations show that inheritance accounts for most of the variation in the size of these cattle, such environmental differences as do exist playing but little part in the ultimate constitution of these animals. The data show high coefficients of assortive mating. These are particularly noticeable for the hip width, body length and rump length. These coefficients are sufficiently large to indicate the separation of the Jersey cattle into non-interbreeding groups, each of these groups being characterized by a definite type. Study of the residual variation, subject to the control of the inheritance, which is left within the groups shows that it has been much reduced in the cattle of any one herd. The individual owners have consequently utilized to nearly its full measure the inheritance which was present initially within the breed. Further selection within the herds consequently must be devoted to the more refined points, or if extensive changes in type are desired resort must be had to crosses between families of the desired types.

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# CATTLE INHERITANCE. I. COLOR<sup>1</sup>

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## INTRODUCTION

A considerable amount of work has been done during the past fifteen years in the study of cattle inheritance. Each worker has taken up only a small portion, and no one has attempted to weld the whole mass into a compact whole. In this, and two other papers which are to follow, such an attempt will be made. The present paper discusses color inheritance, the second will deal with anatomical characters in general, and the third with milk production.

In most of the published papers the effects of each gene have not been carefully defined. This naturally has led to confusion. The following pages will contain as nearly as possible an exact description of the effects produced by each gene, together with its interactions, so far as they are known, with other genes. There probably is no truly logical order of gene presentation, but one sequence may lead to greater clarity than another. The one that seems most satisfactory will be followed here.

The present series of papers is not intended to be to any extent historical, nor will much space be devoted to a discussion of the relative merits of conflicting theories. The main purpose is to outline the present status as it appears to the author. In the course of this presentation hitherto unpublished facts will be given, and factors will be postulated and described which have not as yet appeared in print. The complete, supporting data will not be presented here, but will be published elsewhere when time permits.

## COLOR GENES

In the course of the present paper postulated color genes occupying nineteen different loci will be described in detail. Some of those included are given with a certain degree of hesitancy due to the meager evidence in support of them. Others, not included among the above, but proposed by various authors, will be mentioned chiefly to demonstrate that they do not actually exist. Each of the genes will be designated by means of distinctive letters, in order to avoid confusion; but, to prevent duplication, the letters proposed by the author making the first description of the character will not always be used.

*Red, R*

So far as we know, red (*R*) is present in all cattle. It does not always show because there are so many other genes epistatic to it. We shall therefore have to assume for the present that it is always found in the homozygous condition, and consequently has no allelomorph. It causes the hairs to be red, and, when unaffected by other genes, is responsible for the brownish pigment in the skin of the nose and of the eyelids.

*Black, B*

The gene for black (*B*), found in Aberdeen-Angus and Holstein-Friesians, causes all the pigmented hairs on an animal to become black. Besides having this effect, it is also responsible for the black pigment in the skin, hoofs, tongue, lining of mouth, eyelids, nictitating membranes and "whites" of the eyes, provided the white spotting gene (*s*) does not interfere. The relationship between *B* and *s* will be taken up when the latter factor is discussed.

All that can be said concerning the allelomorph of black, *b*, is that it is the absence of *B*. Animals that are *bb* are not necessarily red, although, as will be shown directly, this represents in part the composition of a truly red animal.

*Black Spotting, Bs*

Black spotting (*Bs*) refers to the type of black found in Jerseys and Ayrshires, and presumably also in Brown Swiss. All the pigmented hairs are not black, and the black is concentrated in certain areas, forming variable sized spots with a somewhat indefinite outline. Since the black is not completely extended over the pigmented part of the coat, red, which is always present, has an opportunity to show. Even in the black areas the hairs may not be entirely black. Besides having the above mentioned effect on the hairs, the *Bs* gene, like the *B* gene, causes black pigment to appear in the skin, hoofs, nose, tongue, lining of mouth, eyelids and "whites" of eyes, if the animal is a self (*S*).

Another peculiarity of the *Bs* factor is that it is not fully expressed in the newly-born calf. Black pigment may be in the tongue and other parts above enumerated, but the black pigment in the hair creeps in gradually with increasing age, and males as a rule develop more black than the females. This sex-limited tendency will be discussed more fully under the factor pair *M* and *L*.

All that can be said concerning the recessive gene, *bs*, is that it is the absence of *Bs*. Since both *B* and *Bs* produce black hairs in one form or another, and since red (*R*) is always present, a *bb bsbs* would be red, with no black hairs showing, unless other genes exist which cause black pigment to appear in the hairs. A *bb bsbs* animal should in addition be devoid of black pigment in the skin, hoofs, nose, tongue, lining of mouth, eyelids and "whites" of the eyes, provided it does not carry the gene *Ps* for pigmented skin. This gene will be taken up later. The Guernsey breed is usually of the composition *bb bsbs*.

When Angus (*BB*) are crossed with Jerseys (*BsBs*) the offspring are black like the Angus, thus demonstrating that *B* is epistatic to *Bs*. Particulars concerning the *F<sub>2</sub>* of such a cross will be given after the factor for

brindling (*Br*) has been discussed. The interactions of *B* and *Bs* and their allelomorphs are as follows:

*BB BsBs*, black

*BB bsbs*, black

*bb BsBs*, black-and-red

*bb bsbs*, red

PEARL (1913) and HOOPER (1921) have postulated a separate dominant gene for black tongue color in Jerseys. The former states in a footnote that further studies indicate that tongue color depends upon two separate factors which show partial coupling on a 3:1:1:3 basis. Both base their conclusions on herd book records. Nevertheless, there seems to be no question but that in at least the great majority of the cases black tongue in Jerseys is due to the *Bs* factor, and not to a separate gene. HOOPER states that of the one thousand descriptions he examined in the registry books of the American Jersey Cattle Club there were only fifteen of self-colored animals that had light tongues and red switches. Such animals were probably *bb bsbs*. There is another more important cause for light-colored tongues in Jerseys and that is the white spotting gene (*s*) which brings about a lack of pigment in the tongue as well as in other parts of the body. The action of the *s* gene will be discussed more in detail later.

FUNKQUIST (1920) assumes that three pairs of factors govern the "muzzle" color of the Swedish breed of Stjernerund. The appearance of the pigmented hairs of this breed is not given, nor is any statement made concerning white spotting. Until one has more particulars concerning the whole animal it does not seem advisable to accept FUNKQUIST's three factor pair hypothesis.

#### Black Spotting Modifiers, *M* and *L*

In 1916 WENTWORTH reported on a sex-limited relation in the Ayrshire breed. He classified all individuals studied into either red-and-whites or black-and-whites and stated that heterozygotes were red-and-white if they were females and black-and-white if they were males. Such an explanation does not fit in with the factorial presentation given in this paper, and I shall endeavor to point out where it seems to be in error. In the first place there are black-and-white Ayrshires in Scotland that carry *B*, the factor causing all pigmented hairs to be black (KUHLMAN 1915b). There is no sex-limited effect here since all heterozygotes are black whether they are males or females.

The gene actually concerned is *Bs*, or black spotting. This factor is found in most Ayrshires and Jerseys, and, as before stated, the males in both breeds show more black than the females. If there were any sex-limited genes they would be modifiers of *Bs*. I have examined a fairly

large number of Ayrshires and Jerseys and find it impossible to separate them into two or three distinct classes, which should be possible if there were only one pair of factors affecting the amount of black that *Bs* produces in the hairs. There is a great deal of variation nevertheless and the males are on the average distinctly darker than the females. The sex-limited effect is present, but it seems to be due to more than one pair of modifiers. Since it is not known how many factors are concerned, it seems advisable to assume at least one pair for the time being. These allelomorphic genes are *M*, much black, and *L*, little black. *M* is dominant in the male and *L* in the female. A heterozygous male (*ML*), therefore, should show much black and a heterozygous female little black.

WENTWORTH described the sex-limited condition in Ayrshires only, but it applies equally as well to Jerseys. LUSH (1929) has recently written a paper, "Atavism in Jersey Cattle," which can be explained by the above interpretation. The case presented by him is as follows: A Jersey cow showing very much black (No. 47) was mated to a bull having very little black and produced a female offspring (No. 10), who from her description and photograph seems devoid of black, therefore being *bsbs*. The latter when mated to a bull showing much black produced a female (No. 34) as black as the granddam (No. 47), thus demonstrating atavism.

The following genetic interpretation is proposed:

♀ No. 47, *Bsbs MM* × ♂, *Bsbs LL* = ♀ No. 10, *bsbs ML*.

♀ No. 10, *bsbs ML* × ♂, *BsBs ML* (or *MM*) = ♀ No. 34, *Bsbs MM*.

*M* or *L* have no effect in the cow No. 10, since it is assumed that they act only in the presence of *Bs*.

There is another sex-limited effect in cattle which may possibly be due to the same modifiers that are responsible for the differences in the amount of black in Ayrshires and Jerseys. It is most noticeable in the red (*bb bsbs*) breeds, such as the Guernseys, Shorthorns and Herefords. In all of these breeds it is usual for the male gradually to attain a deeper shade of red than the females, as maturity is reached. The details concerning these changes have not as yet been investigated. If the same sex differences are present in the black breeds, such as the Aberdeen-Angus and the Holstein-Friesian, they are at least not so apparent.

#### *Brindle, Br*

Brindle usually does not occur in any of the pure breeds of cattle in America, but is found in scrubs, and has been reported among the  $F_2$  generation of the Angus-Jersey cross and also among the  $F_1$  generation of Jersey × Red Danish. There are a few cases known in purebred Ayrshires. The character itself consists of irregular, narrow stripes of black hair on a background of red.



Various attempts have been made to explain the inheritance of this character, but none are completely satisfactory. The most important contribution has been made by COLE (1925), who states that "it may be carried both in full blacks, such as Angus, or in clear reds, such as Red Danish, without being apparent. . . . It would appear to need a partial extension factor [*Bs*] to permit its expression; in the Red Danish such a factor is lacking, and in the Angus the brindling is masked by the action of a factor for the full extension of black [*B*]."

The explanation herein presented, which is essentially the same as COLE's, is that the character, brindle, is due to the joint action of two genes, *Bs* and *Br*. The latter may be looked upon as a pattern factor which causes the black of a *Bs* animal to form into stripes. *Br* has no effect in a *B* animal, since *B* is epistatic to it, and none in a *bb bsbs* animal because there is no black present. Such an explanation fits in with the results obtained by PARLOUR (1913) and COLE (1924) in the  $F_2$  of the Aberdeen Angus-Jersey cross. We would have to assume that many Aberdeen-Angus cattle carry the *Br* factor either in the heterozygous or the homozygous condition, and that many of them are homozygous for *bs*. For the sake of simplicity we shall make both breeds homozygous for all the genes concerned:

$$\begin{array}{lcl}
 \text{Angus (black)} & \text{Jersey (black-and-red)} & \text{(black)} \\
 BB \ bsbs \ BrBr & \times \ bb \ BsBs \ brbr & = Bb \ Bsbs \ Brbr
 \end{array}$$
  

$$Bb \ Bsbs \ Brbr \times Bb \ Bsbs \ Brbr = \left\{ \begin{array}{l} 27 \ B \ Bs \ Br^2 \\ 9 \ B \ Bs \ br \\ 9 \ B \ bs \ Br \\ 3 \ B \ bs \ br \\ 9 \ b \ Bs \ Br, \text{ brindles} \\ 3 \ b \ Bs \ br, \text{ black-and-reds ("Jerseys")} \\ 3 \ b \ bs \ Br \\ 1 \ b \ bs \ bs \end{array} \right\} \begin{array}{l} 48 \text{ blacks} \\ \\ \\ \\ \\ 4 \text{ reds ("Guernseys")} \end{array}$$

Both investigators also describe in the  $F_2$  a "very dark type of Jersey" (COLE 1924), and a black female "with fawn showing through" (PARLOUR 1913). The two descriptions seem to fit the same type of animal, and I shall attempt to explain it genetically when the factor *w* is discussed.

WRIEDT (1919) states that in the Telemark breed of Norway the only colors are brindle and red, and that brindle is dominant. To make these results agree with the factorial explanation given in this paper one would

<sup>1</sup> These represent the various factorial combinations in the  $F_2$  generation. They are therefore zygotes, but the homozygous and heterozygous dominants are both represented by only one letter, as for example, *B*, and the recessives are also represented by means of only one letter (*b*, *bs*, and *br*).

have to assume that many of the reds in the Telemark breed carry *Bs*. WRIEDT gives no details concerning the appearance of these animals.

*Intensity and Dilution, I, i; D, d*

There seems to be good evidence that in cattle there is a form of dilution due to a dominant gene (*D*) and also another form due to a recessive gene (*i*). The former seems to be more pronounced in its effect, especially on black. A black animal carrying *D* is a dun. I have never seen such an animal and hence cannot from personal experience give a definite description of its appearance. It does not occur in any of the pure breeds of North America.

The recessive dilution factor (*i*) occurs in the dairy breeds, Jerseys and Guernseys, and is at least one of the causes for their being a lighter shade of red than such breeds as the Herefords, red Shorthorns and Ayrshires. There is no question but that there must be a number of genes responsible for the extreme variation in shade of red in the different breeds. The specific effect of each has not been determined, nor can one even say what is the precise effect produced by *i*.

If one examines the breeds carrying *B*, such as the Holsteins and Angus, he will find comparatively little variation in shade of black. It does not seem probable that this is because they are relatively homozygous for the dilution factors or their allelomorphs. The true explanation probably is that the dilution factors (except *D*) have less noticeable effect on black than they do on red. The best test for this hypothesis would be to determine the variation in shade of red in the self reds or the red-and-whites that are occasionally produced in the above black breeds.

The inheritance of dun has been studied in West Highland cattle by WATSON (1921). There is no question but that it is due to a dominant factor. However, WATSON is slightly sceptical of WRIGHT's (1917) interpretation that it is the result of the dominant dilution factor (*D*) acting on black (*B*). He states that the results he has obtained can be explained equally as well by assuming that dun is due to a dominant color gene, and that when this gene is present the animal is a dun whether or not it carries black. Supplementary evidence strongly favors the interpretation given by WRIGHT. In the first place, yellows (dilute reds) as well as reds are found in the West Highland breed, which is what would be expected if there were a dominant dilution factor present. In the second place, red and black seem to be the only colors present in cattle, and chocolate seems to be the only other color found in mammals. Cattle would be unique if dun were present as a distinct color. WRIGHT (1917) is of the opinion that *D* is incompletely dominant to its allelomorph.

PRIT (1920) has furnished somewhat meager evidence that *D*, or some

other factor having a similar effect, is present in the Hereford breed. She classifies her animals into "yellows" (light reds) and "clarets" (dark reds) and states that clarets tend to breed true, while yellows mated to clarets produce both yellows and clarets. She has no records of the matings of yellows to yellows because yellow was not a popular shade in the herd from which she obtained her data. The mating together of heterozygous yellows would have been the crucial test.

The results of POUND (1928) conflict somewhat with those of Miss PITT. He finds no true breeding shade of red, although he, like Miss PITT, did not mate yellows to yellows. His yellows seem to carry an incompletely dominant factor. If yellow Herefords actually carry *D* they should produce duns when mated to intense blacks.

I have been informed recently by Mr. E. N. WENTWORTH that many such crosses have been made on the ranges of the Southwest in the United States, and that all the offspring have been intense blacks, thus indicating the *D* is not carried by yellow Herefords.

#### *Self and Recessive White Spotting, S, s*

A self animal is entirely pigmented, and therefore shows no white spotting. The gene for self, *S*, is allelomorphic with *s*, the gene for recessive white spotting, but supposedly *S* is not completely dominant to *s* (COLE 1924). Part of this apparent incomplete dominance may be due to the fact that the ostensibly recessive white-spotted animal may also be carrying genes for dominant white spotting. The question will be discussed more in detail when the latter factors are being considered.

The gene *s* is quite variable in its expression due to modifying factors and also because of non-germinal variation. It seems reasonable to assume that if all variation in this character were germinal, the white spotting on the two sides of an animal, should be exactly the same. Since the two sides are generally unlike we would have to look upon the differences as being non-germinal in nature. DUNN, WEBB and SCHNEIDER (1923) have found a high correlation ( $r = .97 \pm .002$ ) for the amount of black on the two sides of Holsteins. This, of course, does not take into consideration the distribution of the black and white areas.

One point concerning recessive white spotting which previously has not appeared in the literature is that *s* is responsible not only for white areas in the coat but also for unpigmented areas in the tongue, nose, lining of mouth, eyelids, nictitating membranes and "whites" of the eyes. This becomes apparent especially when the animals carry *B* or *Bs*. We have unpublished data (obtained by H. W. MATHEWS and R. R. OEHMCKE) showing that of the approximately one hundred animals making up the Holstein and Ayrshire herds at the KANSAS EXPERIMENT STATION all of them

have entirely unpigmented tongues, while the extent of the unpigmented areas in the other parts above enumerated is fairly closely correlated with the amount of white in the coat.

In making a study of spotting in Holsteins, LAUPRECHT (1926) has viewed it from the standpoint of the location of the pigmented areas rather than of the location of the white spotting. He postulates six paired pigment centers: 1. Eyes and cheeks; 2. Ears and neck; 3. Side of neck; 4. Upper arm and shoulders; 5. Side of body near last rib, and 6. Rump. He states that in certain cases the individual spot may be broken into several parts, or certain spots may be fused. The total amount of black is correlated with the amount at each center. If one examines a series of animals having progressively less pigment, he states that the pigment disappears first from the shoulders, then from the sides and finally from the rump, leaving pigmented areas only on the anterior end of the animal.

Since the factor *s* refers to white spotting it seems less difficult to view its action from the standpoint of the location of the white areas. I have not made a careful investigation of the matter, but my observations so far as they go tend to show that, in animals having only a small amount of white, the location of it is mostly on the limbs and the ventral side of the body. Animals having a large amount of white are white ventrally and posteriorly and to a less extent anteriorly. This last observation accords with that of LAUPRECHT.

#### Modifiers of *s*; *Lw* and *lw*, *Pl* and *pl*

Both LAUPRECHT (1926) and DUNN, WEBB and SCHNEIDER (1923) seem agreed, although they have expressed themselves somewhat differently, that a single pair of modifiers is to a large extent responsible for the varying amounts of white in Holsteins. These genes seem to have nothing to do with the pattern, but are entirely quantitative in their action. DUNN, WEBB and SCHNEIDER have furnished evidence that the factor causing a small amount of white (*Lw*) is incompletely dominant to its allelomorphs (*lw*). Hence animals carrying either a small amount of white or a large amount should breed true. LAUPRECHT maintains that such is the case in Holsteins. Confirmatory evidence may be obtained from other breeds in this country. Guernseys and Shorthorns almost invariably have a small amount of white spotting (*LwLw*) and seem to breed true without selection. Red-and-white Shorthorns, however, have even less white than do Guernseys, an indication that there may be other factors influencing the amount of white. On the other hand there are many Ayrshires that are almost entirely white (*lwlw*). I have looked over a fairly large number of matings in the KANSAS EXPERIMENT STATION Ayrshire herd, where both parents were largely white, and the offspring were similar to the

parents. Such animals tend to be unpigmented not only in the tongue but also on the nose, in the lining of the mouth, the eyelids, nictitating membranes and the "whites" of the eyes.

Another modifier of *s* has recently come to light (IBSEN and RIDDELL 1931). Animals carrying it, *Pl*, are pigmented to a large extent below the knees. The factor acts as an inhibitor of white in these regions. It also causes some irregularity of the white spotting on the head by adding small pigmented areas. As before indicated, the legs below the knees are not entirely pigmented, and the extent of the white seems to be somewhat correlated with the amount of white in other parts of the body. In "pigmented-legged" (*Pl*) animals the pigmentation seems to be concentrated around the hoofs and to extend upward, and is easily distinguished from cases where a pigmented spot spreads downward past the knee. The gene *Pl* seems to be found only in Ayrshires and Shorthorns in this country. Holsteins, Guernseys and other breeds are *plpl* and therefore one would expect them to be entirely white from the hoofs to the knees. There are a number of exceptions however. For example there are Holsteins with one or more legs entirely black. Oftentimes such animals have very little white and this may account for some of the cases. In others this is not a satisfactory explanation, and as yet no examination of the ancestry or of the progeny has been made to determine whether there is any evidence of the character being hereditary. Another exception occurs in Guernseys where a fairly large number of individuals are found with small pigmented patches directly above one or more hoofs. These patches are probably not due to the *Pl* factor, but whether or not they are inherited has not been determined.

FUNKQUIST and BOMAN (1923) postulate three pairs of genes affecting the amount of white on the heads of Holsteins and a red-and-white Swedish breed. In their paper they do not take into consideration the white spotting on other parts of the body. They postulate that an entirely pigmented head is due to the joint action of three recessive genes, and an entirely white one to at least two homozygous dominants plus either a homozygous or a heterozygous third dominant.

The facts, so far as I have been able to ascertain them in American Holsteins, are not in accord with this theory. Occasionally animals are found with heads almost entirely white even though the remainder of the body may be largely pigmented. One dominant gene is responsible for this condition, although the evidence as yet is not by any means complete. It is uncertain whether the gene is a modifier of *s* or is one for dominant white spotting.

Phenotypically, from the standpoint of the location of the white areas, a white-faced Holstein of the above description resembles typical speci-

mens of the Swiss Simmental breed. In Germany Simmentals are called "Fleckviehs," and LANG (1914) records a cross of this breed with a self-colored (*S*) breed known as the Limburger. The  $F_1$  were pigmented except for their white faces, thus showing that the Fleckviehs carry a gene for dominant white spotting. It will be shown later that this gene is distinct from that producing the Hereford pattern. There is no record as yet of crosses between white-faced Holsteins and self-colored breeds.

Most Holsteins do not carry the dominant gene for white head, and yet there is much variation in the amount of white spotting in that region. There is no question in my mind but that for those animals lacking the dominant gene there is a close correlation between the amount of white on the body and the amount on the head. Animals of the composition *lwlw* have more white on the head than those that are either *Lwlw* or *LwLw*. This would be directly contrary to the theory of FUNKQUIST and BOMAN who postulate dominant genes for increased white spotting.

Occasionally pure white, or almost pure white, Ayrshires and Holsteins are found. Such animals are *ss lwlw* to begin with, and probably also carry "minus modifiers." The existence of the latter has not as yet been proved for this particular case. At any rate, animals that are practically white would have white heads which are due to the combined effect of several genes, some of which are known to be recessives.

HOOPER (1921) assumes there is a separate gene for white switch in Jerseys. He obtained his data from the registry books of the AMERICAN JERSEY CATTLE CLUB. An analysis of the data shows that the white switch was found mostly in animals that carried white spotting (*ss*) in other parts of the body. A few so-called selfs also had white switches. My observations lead me to believe that a white switch is only one of the manifestations of the *s* factor, and that, if an animal carries any white at all, the end of the tail is one of the places almost invariably affected. I have already called attention to the fact that the same applies in regard to the effect of *s* on the pigmentation of the tongue—animals that are *ss* almost invariably have unpigmented tongues.

### *Roan, N*

The mode of inheritance of roan in Shorthorns and in crosses between Shorthorns and other breeds has attracted a great deal of attention in recent years. A number of different theories have been advanced, but it would require too much space to give all of them in detail.

The simplest, and probably the first, explanation given is that red and white are allelomorphic and that red roan is the heterozygote. One difficulty with this theory is that it cannot be applied satisfactorily to crosses between white Shorthorns and black breeds, such as the Angus. The  $F_1$

from such a cross are "blue grays" and at first glance one might suppose that black also is allelomorphic with white. If such were the case the  $F_2$  generation should consist only of whites, blue grays and blacks. There are actually two kinds of whites—those with red "points" and those with black, and, besides the blue grays and blacks already mentioned, red roans and reds. The results in the  $F_2$  indicate that we are dealing with a dihybrid cross instead of a monohybrid. An apparently new and at the same time satisfactory explanation of the above results was devised, but it was found later that it had been antedated a number of years by CREW (1925). His theory as given in his own words (p. 68) is as follows: "All Shorthorns may be reds. If this is so, it is necessary only to postulate a series of modifying factors affecting (1) the extension or restriction and (2) the regional distribution of the colour to get roans and also parti-coloured beasts. A white would thus be duplex for the restriction, a roan one that is simplex. The factors affecting regional distribution [*s*] can act on a roan as well as on a solid colour."

CREW's explanation is not quite as specific as the one I had in mind. In the latter the incompletely dominant factor *N* is postulated, which causes hair of any color to become devoid of pigment and therefore white. In the heterozygous condition (*Nn*) approximately one-half of the hairs interspersed throughout the pigmented areas are white, causing the animal to become a roan, while in homozygotes (*NN*) nearly all the hairs except some in the ears are white. Such an explanation can be readily applied to the white Shorthorn-Galloway cross reported by EVVARD, SHEARER, LINDSTROM and SMITH (1930), and seems more satisfactory than the one they themselves propose.

In their paper they put forward the following factor pairs to explain their results:

*B* = black.

*b* = red.

*E* = extension factor, causes pigment to cover whole coat.

*e* = absence of extension factor, causes pigment to be found only in points.

*N* = roan (sprinkling of white hairs) completely dominant to *n*.

*n* = no roan.

Using these factors, they designate a white Shorthorn as *bb ee NN* and a Galloway as *BB EE nn*.

Their theory seems quite inadequate even when applied to crosses within the Shorthorn breed. For example, if a white Shorthorn (*bb ee NN*) is mated to a red (*bb EE nn*), assuming both to be homozygous, the  $F_1$ 's will be roans (*bb Ee Nn*), as would be expected, but in the  $F_2$ 's there will be

many unusual genotypes. The  $F_2$  phenotypic ratio should be nine roans: three reds: four whites, but of the roans one ( $EE NN$ ) should be entirely homozygous, two ( $EE Nn$ ) should produce no whites when inbred, two ( $Ee NN$ ) should produce no reds when inbred, and the remaining four ( $Ee Nn$ ) should breed like true roans and produce reds, whites and roans when inbred. Of the three reds in the  $F_2$ 's, two ( $Ee nn$ ) should produce whites when inbred, and one of the four whites ( $ee nn$ ) should produce no roans when mated to reds.

If we use the genes suggested in this paper, remembering that my  $N$  is different from theirs and that a  $bb$  animal is also assumed to be  $bsbs$ , the crosses would be represented as follows:

$$\begin{aligned} \text{White } (bb NN) \times \text{red } (bb nn) &= \text{Roan } (bb Nn) \\ &\quad bb NN \text{ (white)} \\ bb Nn \times bb Nn &= 2 \text{ } bb Nn \text{ (roan)} \\ &\quad \quad \quad bb nn \text{ (red)} \end{aligned}$$

It is true that there are occasional exceptions. For example there are cases known where whites mated to reds produced reds instead of roans, but, if the interpretation of EVVARD and co-workers were correct, these would not be exceptions. They would be far too numerous to come under that classification. I shall attempt later to explain the exceptions.

Using the genes suggested above, the white Shorthorn-Galloway cross may be represented in the following manner:

$$\text{White Shorthorn } (bb NN) \times \text{Galloway } (BB nn) = Bb Nn, \text{ blue gray}^3$$

$$Bb Nn \times Bb Nn = \begin{cases} \left\{ \begin{array}{l} NN, 3 \text{ whites, black points} \\ 3B \left\{ \begin{array}{l} 2Nn, 6 \text{ blue grays} \\ nn, 3 \text{ blacks} \end{array} \right. \end{array} \right. \\ \left\{ \begin{array}{l} NN, 1 \text{ white, red points} \\ 1bb \left\{ \begin{array}{l} 2Nn, 2 \text{ red roans} \\ nn, 1 \text{ red} \end{array} \right. \end{array} \right. \end{cases}$$

EVVARD and co-workers obtained the following offspring in the  $F_2$  generation: 11 blue grays, 5 reds, 3 whites with black points, 1 black, and 1 red roan, or a total of 21. Their numbers are small. Using HARRIS'S formula for goodness of fit and ELDERTON'S table, for the interpretation proposed above, the probability (P) was found to be .0061.

In their  $F_2$  generation EVVARD and co-workers obtained a number of animals that showed recessive white spotting ( $s$ ). Since the  $P_1$  Galloway

<sup>3</sup> The term "blue gray," employed by EVVARD and co-workers, represents conventional usage. From a factorial standpoint it would be more appropriate to designate such animals black roans, a term more easily contrasted with red roan.



cows and the  $F_1$  blue grays were selfs ( $S$ ), it would mean that the  $P_1$  white Shorthorn bull must have been  $ss$ , which would not be apparent phenotypically because  $N$  in the homozygous condition is epistatic to  $s$ . The white Shorthorn bull thus would be  $bb\ NN\ ss$  and the black Galloway cows  $BB\ nn\ SS$ . Taking white spotting into consideration, they obtained 7 different phenotypes among their 21  $F_2$  animals. Theoretically there should have been 10 phenotypes. What seems rather remarkable is that when one determines  $P$  for their  $F_2$  phenotypes, using again the factorial interpretation proposed in this paper, it is found to be .0371. The details have not been given due to lack of space.

### Roan Modifier, *rm*

The main objection that EVVARD and co-workers have to other explanations than their own is that they do not explain exceptions. I have already pointed out that with their own explanation one would expect many more exceptions than actually occur. I have also mentioned that the chief exceptions are cases where white bulls, such as Whitehall Sultan, have produced some red offspring when mated to red cows. The figures for Whitehall Sultan as given by EVVARD and co-workers are 44 roans and 15 reds. Whitehall Sultan's son, Maxwalton Sultan, also white, produced 41 roans and 13 reds when mated to red cows.

SMITH (1925) reports the case of a bull that was a red roan when young which gradually lost his white hairs and became a red. This might possibly explain some of the reds produced in the white  $\times$  red matings, but it undoubtedly would not explain all of them. One reason for this is that most purebreds are registered when young.

I am offering a tentative explanation for the above exceptions by postulating a new recessive modifying factor, *rm*. An animal of the composition  $bb\ Nn\ rmr m$  would by this hypothesis be red because *rm* (roan modifier) changes roan to red. A typical roan would be either  $bb\ Nn\ RmRm$  or  $bb\ Nn\ Rmr m$ . The mating of a white bull like Whitehall Sultan to red cows could be represented in various ways. White bulls would be either  $RmRm$ ,  $Rmr m$ , or  $rmr m$  without any phenotypic effect, the one possible exception being that a  $NN\ rmr m$  animal might be a roan instead of a white. If this were the case it would be possible to produce a true-breeding strain of roans. Whitehall Sultan should be  $Rmr m$  at least, and the red cows that had red offspring by him should be either  $Rmr m$  or  $rmr m$ . Since the occurrence of *rm* in Shorthorns may be considered rather infrequent, most of the matings of a bull like Whitehall Sultan would be with  $RmRm$  red cows, and

( $RmRm$ )

the offspring from such matings would be  $bb\ Nn$  ( or ), or roans.

( $Rmr m$ )

Although there is no definite proof of the existence of the factor *rm*, there are various ways in which a test could be made for it. The exceptional red cows produced in the white  $\times$  red mating should be *bb Nn rmm*. When mated to ordinary whites (*bb NN RmRm*) they should produce whites (*bb NN Rmm*) as well as roans (*bb Nn Rmm*), and when mated to ordinary reds (*bb nn RmRm*) they should produce roans (*bb Nn Rmm*) as well as reds (*bb nn Rmm*). It does not seem extravagant to propose a modifying factor like *rm* when one considers the great variation in the expression of the roan character in Shorthorns and the modifiers that are undoubtedly responsible for this variation.

One more point that probably should be made in regard to the interaction of *N* with other genes is that when a white Shorthorn (*bb bsbs NN*) is mated to a Jersey (*bb BsBs nn*) the offspring (*bb Bsbs Nn*) should be blue gray in spots and red roan on the remainder of the body if there is no white spotting present. I have seen animals that fill this description, but their parentage was unknown.

#### *Recessive White, Wn*

MANRESA, GONZALEZ, SARAO and ESGUERRA (1930) report on some crosses made in the Philippines between Native cattle and either Herefords or the Nellore breed of India, and also between Herefords and Nellores. Their description of the Nellore breed is as follows: "Nellores are phenotypically white, but indications are that they are really gray. White animals have unpigmented skins. The skins of Nellores are usually deeply pigmented, that is, almost black. For lack of a better term we will call the color of Nellores silver gray."

When Herefords were crossed with Nellores three types of offspring were produced, (1) brindled, (2) black, and (3) "dark fawn, with black extremities." The authors do not attempt to explain the inheritance of the Nellore's "silver gray" in the above cross, but do so in the cross between Native cattle and Nellores, where they obtained some "grays." They assume that "silver gray" is due to a dominant gene, designated by them *A*, which in the homozygous condition is completely epistatic to both the black and the dominant dilution factor (*D*) of the Native cattle, while it is incompletely epistatic when heterozygous.

It seems more logical to base the inheritance of "silver gray" on the results obtained when Nellores are crossed with Herefords, since we are better acquainted with the genetic make-up of the latter breed than of the Native cattle of the Philippines. If this is done, one is forced to conclude that the "silver gray" of the Nellores is actually a form of recessive white, the factor for which can be represented by *wn*, the *n* standing for Nellores, and thereby distinguishing this from other forms of recessive white which

may occur. The statement by MANRESA and co-workers that white animals have unpigmented skins is true, but their implication that they are no longer white if the skin is pigmented does not hold. White Silkies, a breed of domestic fowl, have pigmented skins and yet are considered to carry a factor for recessive white. It may also be (I have not had an opportunity to make a personal inspection) that the whites with black points produced in the  $F_2$  of the White Shorthorn-Galloway cross have black skins. If the authors had said that true albinos do not have pigmented skins, it would not have been questioned.

The fact that Nellores when crossed with Herefords produce brindles, blacks and "fawns" demonstrates that this Indian breed carries  $Bs$  and sometimes  $B$ . The results from all three of their crosses agree with the assumption made by them that the brindling factor ( $Br$ ) is carried by the Herefords, although there is still the possibility that it is also carried by the Nellores.

Several possible explanations may be given for the black skin of the Nellores. One is that it is due to a separate factor. Another is that  $wn$  is incompletely epistatic to either  $Bs$  or  $B$ . Both of these factors cause the production of black in the skin as well as in the hair and other parts of the body, and the  $wn$  gene when homozygous may prevent the formation of pigmentation in the hair, but have no effect on the skin.

Information received recently from Mr. E. N. WENTWORTH bears out the above interpretation. Shortly after the War he made a microscopic examination of white hairs from several Nellore cattle exhibited at the Fort Worth, Texas, Show. Even macroscopically the hairs did not seem a pure white, and this was borne out under the microscope by the fact that black and red pigment granules were found sparsely scattered throughout the hair. Based on the above observations, it seems logical to interpret the  $wn$  gene as one causing extreme dilution. The presence of both black and red pigment granules in the hairs indicates that the animals investigated were  $bb$ , but at the same time carried  $Bs$ . It also seems reasonable to assume that since Nellores carry either  $B$  or  $Bs$ , or both, that the  $wn$  gene causes extreme dilution in the hairs, but has relatively less effect on the pigment of the skin, which consequently remains black.

I have no explanation to offer for the "grays" obtained in many cases when Nellores are crossed with Native cattle unless it be that the Native cattle are often  $Wnwn$ , thus offering opportunity for the outcropping of "silver grays" ( $wnwn$ ) in the offspring.

The foregoing account possibly may be made a little clearer by giving the assumed composition of the Herefords and Nellores, making them heterozygous for certain factors in order to show all the different types of offspring produced:

Herefords                      Nellores

$bb\ bsbs\ Brbr\ WnWn \times Bb\ BsBs\ brbr\ wnwn =$

$$\left\{ \begin{array}{l} Bb\ Bsbs \left\{ \begin{array}{l} Brbr\ Wnwn, \text{ black} \\ brbr\ Wnwn, \text{ black} \end{array} \right. \\ \\ bb\ Bsbs \left\{ \begin{array}{l} Brbr\ Wnwn, \text{ brindle} \\ brbr\ Wnwn, \text{ "fawn"} \end{array} \right. \end{array} \right.$$

KUIPER (1921) makes the statement that a recessive form of white is found in both the Pembroke and the Highland breeds of cattle, but he furnishes no references or proofs. So far as I have been able to determine, the evidence thus far is quite inconclusive. WALLACE (1907) states that the white Pembrokes are "throw backs" from the black breed of South Wales, which would make them appear to be recessive whites, but he also states that when a black bull of the South Wales breed is mated to white Pembroke cows "a large proportion of his progeny take after the mothers in being white with black points." The last statement would best fit in with the assumption that many of the white cows are heterozygous dominants. There is still the possibility, however, that most of the South Wales black bulls are heterozygous for recessive white.

WILSON (1909) has made a genetic analysis of color in the Highland breed. He is of the opinion that white cattle imported by the Romans into England from southern Europe were one of the ancestors of this breed, and that for many years occasional whites occurred amongst them. The fact that no whites appear at present may more easily be explained by assuming that the ancestral white was due to a dominant rather than to a recessive gene.

### *Dominant White, Wp*

It has long been known that the white English Park cattle (Cadzow Castle Park, et cetera) carry a factor for dominant white. WRIEDT (1929) has called attention to this fact recently. He states that the same factor is found in Swedish mountain cattle. Animals carrying the gene are not completely white, but, in addition to having pigmented ears, may have colored flecks on the body. SANDERS (1925) has a photograph of six Cadzow Castle Park cattle. One of these animals is to a large extent hidden by the others, and a few are partially hidden. All six, however, seem to have black "points," four have small pigmented spots on the neck, and five have pigmented areas on the legs which correspond in appearance to those produced by the *Pl* (pigmented leg) factor in Ayrshires.

It would seem from the above that the white of English Park cattle actually is a form of dominant white spotting which causes the animal to

be largely white. I have called the factor producing it  $Wp$ , the  $p$  having reference to Park. As yet I have not come across any reference to its genetic relations with self ( $S$ ). There are photographs, each showing non-whites in a group of White Park cattle, and these non-whites have all had the appearance of being self blacks. In every case they are said to be offspring of white parents, and, if this is true, it would mean that black ( $B$ ) and self ( $S$ ) are common in these animals and that  $Wp$  is incompletely epistatic to self. A  $wpw p$  animal would merely be one that did not carry dominant white ( $Wp$ ).

### *Dominant White Spotting*

#### Hereford Pattern, $S^H$ , $S, s$

The typical modern Hereford is red with a white head, white feet and tip of tail, white stripe of varying width and length over and parallel to the backbone in the region of the shoulders, and with white on the entire ventral side of the body. It has been customary to assume that the inheritance is very simple,  $H$  generally being used to designate the factor for the white spotting and  $h$  its absence. No discussion that I have seen has ever considered the relation of the above factor  $H$  to either  $S$  or  $s$ . When one takes this relationship into consideration, there are two main possibilities as to the part which  $H$  plays in the production of the whole Hereford coat pattern, under which heading would be included the pigmented as well as the white areas.

One possibility is that  $H$  is responsible for both the white and the pigmented areas and their distribution, and is a dominant allelomorph of  $S$  and  $s$ . Instead of being allelomorphic, it could be epistatic to these factors; but this will not be considered for the time being. The above explanation would account for the apparent ease with which the Hereford pattern has been fixed, since it would be necessary to have the animal homozygous for only one factor. On the other hand it would not explain the genetic composition of the "Fleckvieh" (Simmental) breed of Germany, which in addition to a white face and white extremities, also has white in places found only on a  $ss$  animal (LANG 1914). There is no evidence available, however, to show that the dominant factor which produces white face in Fleckviehs is the same factor that is responsible for white face in Herefords.

If we assume an allelomorphic relationship between the three factors it could be represented as follows, the factors being given in their order of dominance:

$S^H$  = Hereford pattern.

$S$  = self.

$s$  = recessive white spotting.

There is evidence that  $S^H$  is incompletely dominant to  $S$  and completely dominant to  $s$ . This is shown by the fact that when Herefords are crossed with either Angus or self Jerseys the offspring have little or no white on the legs and less white on other parts of the body than the Hereford parent. When they are crossed with white-spotted ( $ss$ ) breeds like the Holsteins the offspring show no diminution of white.

Some evidence obtained recently from the herd of Mr. JOHN SHOEBOOK of Atchison County, Kansas, fits in well with the allelomorphic conception. He crossed purebred Herefords ( $S^H S^H$ ) with high grade white spotted Shorthorns ( $ss$ ), mated the crossbreds to Herefords, and continued backcrossing to Herefords for a number of generations. Recently he took one of the Hereford-marked bulls that he had produced as a result of the above sort of breeding and mated him to similar cows. Most of the offspring had typical Hereford markings. There were only two exceptions, and both were intermediate white-spotted individuals ( $ss Lwlw$ ). From the results obtained, on the multiple allelomorphic interpretation, it would mean that the bull and at least two of the cows are  $S^H s$ .

The other possibility in regard to the genetics of the typical Hereford pattern is that the factor  $H$  is responsible only for the white spotting and that  $S$  causes the remainder of the coat to be pigmented. In such a case  $H$  would be epistatic to  $S$ , and a true breeding Hereford would have to be homozygous for two factors,  $H$  and  $S$ . This sort of explanation could be applied satisfactorily to the Fleckvieh breed. The latter would be of the composition  $HH ss$ , because of the excessive white spotting appearing on the body. One drawback to a two factor hypothesis for the Hereford coat is that one would expect greater difficulty to have been experienced in fixing it than was actually the case. Even at the present time it should not have been unusual for sporadic cases of self reds, white-faced animals showing recessive white spotting, and others showing only recessive white spotting, to appear.

Of the two explanations proposed I am inclined to favor the multiple allelomorphic one. There is also a third possibility, previously mentioned, that of a factor similar in its effect to  $S^H$  but which is epistatic rather than allelomorphic to  $S$  and  $s$ . All three possibilities could be tested by appropriate matings. What seems to be the most feasible method is to mate Herefords to  $ss$  animals, preferably some homozygous for  $lw$ , and to backcross the hybrids to  $ss lwlw$  individuals. If  $S^H$  is allelomorphic to  $S$  and  $s$ , there will be only two phenotypes resulting from the backcross—"Herefords" and recessive white spotted animals. If it is epistatic, there should be in addition to the above two phenotypes, an occasional self ( $S$ ). If the Hereford pattern is due to the combined effects of  $H$  and  $S$ , the backcross should produce four different phenotypes, with equal numbers of each:

$$HHSS \times hhss = HhSs$$

$$HhSs \times hhss = \begin{cases} HhSs, \text{ Hereford pattern.} \\ Hhss, \text{ Hereford pattern, plus recessive white spotting.} \\ hhSs, \text{ self.} \\ hhss, \text{ recessive white spotting.} \end{cases}$$

It should be evident that if either the multiple allelomorphic or the epistatic explanation proves to be the correct one, it will be necessary to postulate a different dominant factor for the white face of the Fleckvieh breed. I believe it will be found that the gene causing the white face of the Fleckviehs is different from that producing the white face of the Herefords.

$S^H$  Modifiers,  $Lw, lw$ ;  $Rn, rn$ ;  $Re, re$ .

Although we are uncertain as to the true genetic explanation for the Hereford pattern, there is no question but that so far as we know every individual in the breed is homozygous for the gene, or genes, producing this pattern. At the same time there is a great deal of variation in its expression, due to modifying factors. Some interesting, though meager, results have been obtained on this point by Miss PITT (1920).

She looks upon the factors she proposes as allelomorphs to one producing the Hereford pattern, but furnishes no proofs. It seems more appropriate to consider them modifiers of  $S^H$ , and as such they will be discussed. The first is a recessive factor which causes the animals to have an increased amount of white. From her standpoint it is a special factor found in Herefords, but there is some, as yet unpublished, evidence accumulated which justifies one in assuming, at least tentatively, that her factor and  $lw$  are identical. The evidence above mentioned has been obtained from the crossing of Herefords with Holstein nurse cows. The two Hereford bulls used had slightly more white than would be considered desirable, and, judging by their offspring as well as by their appearance, were of the composition  $Lwlw$ . The cows were either  $Lwlw$  or  $lwlw$ . The five calves produced thus far have either had an extremely large amount of white ( $lwlw$ ) or a slight excess ( $Lwlw$ ). No  $Lw Lw$  animals have appeared, but the total number as yet is small. Observations on purebred Hereford matings also indicate the incomplete dominance mentioned above. Miss PITT implies that the dominance is complete. For the present, at least, it seems advisable to assume that  $Lw$  and  $lw$  are modifiers of the Hereford pattern factor ( $S^H$ ) as well as of that for recessive white spotting ( $s$ ).

$Rn, rn$

Miss PITT proposes another modifying factor which causes the entire

dorsal surface of the neck and shoulders to be pigmented. An animal with such a character she describes as "dark-necked," while the Hereford breeders of the United States refer to it as "red-necked." The latter term seems the more appropriate of the two. The factor, which I have designated *Rn*, is incompletely dominant to *rn*, the absence of red neck. Thus, a *Rn rn* animal, according to Miss PIRT, would have a small amount of white dorsally on the neck, and if at the same time it were *lw lw*, the white in that region would be increased so appreciably that it would look like an individual with the normal amount.

Since it has been assumed that *Lw* and its allelomorph are modifiers of both *s* and *S<sup>H</sup>*, it seems desirable to speculate on the possibility of *Rn* being also a modifier of *s*. Some evidence on that point has been obtained from Hereford-Shorthorn crosses. Shorthorn breeders for many years have made an effort to decrease the amount of white spotting in their breed by careful selection. The effect has been quite marked, and as a result Shorthorns have much less white than Guernseys, which as a breed are *Lw Lw*. Many of the Hereford-Shorthorn crossbreeds have red necks and in general an increase in pigmentation, seemingly due to the Shorthorn parent. The evidence, such as it is, fits in with the hypothesis that the Shorthorn breed not only is *Lw Lw*, but in addition carries another dominant modifier of *s*, in this case assumed to be *Rn*.

### *Re, re*

A third modifier proposed by Miss PIRT is responsible for the production of red hair around each eye. Most Herefords have entirely white faces and as a result the eyes of many of them become injured if they are long exposed to the glare of the sun. Those with "red eyes" are less liable to such injury. The factor producing red eye, *Re*, is considered to be completely dominant to *re*, absence of red eye, although Miss PIRT has no definite information on this point. She also has little or no evidence that *re re* animals breed true. POUND (1932), on the other hand, makes the statement that "animals with white at the eye, when bred together, produced practically nothing but calves like the parents." He makes the additional implication that *Re*, instead of always causing red hair to form about the eye, may sometimes produce only some pigmentation in the skin of the eyelids. It seems impossible at present even to guess what effect *Re* would have on a *ss* animal. So far as I have been able to determine, "red eye" is found only in Herefords or in other breeds carrying the *S<sup>H</sup>* gene.

There are many variations in the Hereford pattern and the three pairs of modifiers proposed by Miss PIRT would not by any means account for all of them. Moreover, she does not assume that they do. POUND (1932)



has called attention to one of the more striking variations not discussed by Miss PRRT. It is known amongst breeders as "lineback." Animals with this characteristic are white along the top line, from the head to, or almost to, the tail. Oftentimes it is associated with reduced pigmentation over the entire body, but there are many exceptions to such a rule. It is not known as yet whether or not lineback is due to a separate modifier, but, the fact that two animals lacking the character may produce an offspring that shows it, makes it look as if it were inherited as a recessive. Very few lineback bulls are retained for breeding because of the undesirability of the character.

Amongst early Herefords there were many individuals with "brockle" faces. Such animals had large blotches of pigmented hair on an otherwise white face. At the present time similarly marked animals are found on those ranches of the western United States where there has been some intercrossing between Herefords and Shorthorns. A more careful examination of the pictures of early Herefords and also of the living descendants of crossbreds, shows that all brockle-faced cattle are "pigmented-legged" (*Pl*). There seems to be no question but that the same factor produces both effects. Since the brockle face is due to a dominant gene, no difficulty should have been experienced in eliminating it by selection from the Hereford breed. The *Pl* factor is quite common amongst Shorthorns, and thus furnishes the explanation for the presence of brockle faces amongst the descendants of the Hereford-Shorthorn crossbreds. Such animals are raised only for the market and no selection is practiced in regard to their pattern.

Although it is not intended in the present paper to discuss all of the European breeds, I cannot resist the temptation to take into consideration one that apparently carries the *S<sup>H</sup>* gene. This is the Groningen (called "Groninger" by KUIPER [1921]) breed of Holland.

The observations on Groningens are based on eight photographs obtained from three different sources. All eight animals (three males and five females) have white faces but are pigmented around the eyes (*Re*). These pigmented spots are continuous with the pigment of the neck. The pigment on the head and neck extends further forward than it does in typical Herefords. None of the eight animals have dorsal white stripes. This makes them red-necked (*Rn*). The three bulls apparently have no white on the belly, while all five cows have white udders and an additional small amount of white ventrally on the body (*LwLw*). In some of the photographs the hoofs, and that part of the legs directly above them, do not show distinctly, making it difficult to determine the amount of white in that region. Nevertheless, four animals are white on all four legs. It extends upward only a short distance, however. One bull unmistakably has four entirely

pigmented legs, and a cow has white on both rear legs and none on the forelegs. The location of the white on the legs of the other two animals cannot be determined with certainty.

It is assumed that the eight Groningens discussed represent desirable specimens of the breed. All of them presumably carry two completely dominant (*Re* and *Rn*) and one incompletely dominant (*Lw*) modifier of *S<sup>H</sup>*. Each of these modifiers tends to increase the amount of pigmentation on the animal. If careful selection for increased pigmentation has been practiced, one would expect most of the breed to be *LwLw* because of the incomplete dominance of *Lw* to *lw*; but there should still be a number of individuals heterozygous for either of the other two modifiers, who, when mated, would produce some offspring either with a clear white face (*rere*) or with a white stripe dorsally on the neck (*rnrm*). Critical evidence on the *S<sup>H</sup>* interpretation could also be obtained. If *S<sup>H</sup>* actually is responsible for the Groningen pattern, no white-faced animals showing recessive white spotting (*s*) should be produced.

The Norman breed of France also apparently carries the *S<sup>H</sup>* gene. I have at my disposal at present the photograph of only one individual, and hence will attempt no generalizations. This animal, a bull, is pigmented-legged and brockle-faced (*Pl*), has pigment around the eyes (*Re*) and a dorsal white stripe on the neck (*rn*). It has enough white on the belly to be classified as a *Lwlw* individual. One striking characteristic that differentiates it from Herefords and Groningens is the marked irregularity of outline of the ventral edges of the pigmented area. Similar irregularities of outline are found in Ayrshires and Guernseys, and the evidence from these breeds, based on general observations, is that the character is due to one or more recessive genes.

### *Dominant White Spotting*

#### Dutch Belt, *S<sup>D</sup>*, *S*, *s*

All the published evidence on the inheritance of Dutch belting seems to have been furnished by KUIPER (1920, 1921). The photographs and diagrams in his papers show that the belt, which is white, varies in width and regularity of outline and extends around the body back of the shoulders and forelegs. The character is found chiefly in the breed known in this country as the Dutch Belted Cattle, and in Holland as Lakenvelders. The breed seems to have originated in Holland, and its distribution is not widespread. The typical color pattern is black and white with the white being almost entirely restricted to the belt. The most common exception seems to be a small amount of white directly above the rear hoofs.

KUIPER (1921) states that the off-types in the breed are self blacks, red dutch belted, and self reds, and that these are due to the occasional out-

crossing that has been practiced. It is significant that he does not mention the occurrence of animals which have the typical recessive white spotting ( $s$ ) in addition to the dutch belt, nor of animals having only the recessive white spotting. If the first of these two types never occurs it will have an important bearing on the mode of inheritance of the belted condition.

The explanation as given by KUIPER is that there are two pairs of factors concerned. These are the factor for self ( $S$ ) and the dominant factor for white belt (he uses  $B$  for belt and  $b$  for absence of belt). His factor  $B$  is considered to be epistatic to self. At first glance this seems to be a satisfactory explanation, but, as previously intimated, there is at least one serious objection, which is that, since the breed is to some extent heterozygous, there should be some matings in which both parents are  $Ss$ , and who as a result should produce an occasional  $ss$  animal having in addition a dutch belt. One gets the impression from KUIPER that animals of this description are never produced.

If this is the case, it seems advisable to fall back on a multiple allelomorphous explanation, as was done with the factor for Hereford pattern ( $S^H$ ). One would, under the circumstances, have to assume that a single factor not only produces the white belt but is also responsible for the remainder of the coat being pigmented. The gene having this effect,  $S^D$ , like  $S^H$ , would be allelomorphous to  $S$  and  $s$ , and would be dominant to them. With the factors in an allelomorphous series, it would be impossible for a dutch belted animal to show any recessive white spotting unless  $S^D$  happened to be incompletely dominant to  $s$ . The results recorded in KUIPER's paper show that the dominance is complete. The multiple allelomorphous interpretation would also explain the relative infrequency of recessive white-spotted ( $ss$ ) animals in the Dutch Belted breed. It would be necessary for both parents to be  $S^D$ s before any could be produced. If one parent were  $S^D S$  and the other  $S^D s$ , the off-types would be selfs.

The chief data presented by KUIPER are the results obtained from the mating of a purebred Dutch Belted bull with fifty-three  $ss$  cows, and with one "Groninger Zwartblaard" ( $S^H$ ), cow. Only the  $F_1$  animals were reported on. The bull, according to the interpretation herein presented, was of the composition  $S^D S$ . There were fifty-five  $F_1$  animals. Of these, twenty-seven were belted, "twenty-four or twenty-five" were self-colored, and "three or four" were "pied." Some of the selfs had small white spots. The pied individuals were assumed by KUIPER to be of the composition  $ss$ . The location of the white spotting on two of the latter is shown by means of diagrams, and there is no question in my mind but that they are imperfectly marked dutch belted individuals. They have less white than is found normally in the Dutch Belted breed, but the same amount of white in a  $ss$  animal would be located almost entirely on the belly and the feet,

and would not extend up so far along the sides. I shall venture the guess that their *ss* mothers showed very little white spotting.

KUIPER offers the tentative explanation that the bull was *B(belt)bSs*, and that these factors are linked with approximately 12.5 percent crossing over. There are two flaws in his explanation. One is, as already pointed out, that there are no *ss* animals in the  $F_1$  generation, and the other is that he assumes a *Bbss* animal to be as typically dutch belted as one of the composition *BbSs*. The multiple allelomorphic representation of the cross would be:

$$S^D S \times ss = \begin{cases} S^D s, & \text{dutch belted.} \\ Ss, & \text{self.} \end{cases}$$

If one considers the "pied" individuals as dutch belted, the numbers obtained were approximately thirty dutch belted and twenty-five selfs. A critical test of the correctness of the two theories would be the mating of the  $F_1$  dutch belted to *ss* animals.

Since  $S^D$  and  $S^H$  are assumed to be allelomorphic, it would be of interest to determine the interaction of the two genes in a heterozygote ( $S^D S^H$ ). So far as I know only one such individual has been reported on thus far. It has already been referred to in the detailed account of KUIPER's data. The purebred Dutch Belted ( $S^D$ ) bull was mated to a Groningen cow, belonging to a breed presumably carrying the  $S^H$  gene. The one offspring, as represented by KUIPER in a very small and somewhat indistinct diagram, has the white face of the Groningen mother and the belt of the father. Both hind legs also seem to be white. The two allelomorphic genes affect different parts of the body and both are enabled to express themselves in the heterozygote.

### *Dominant White Spotting*

Colorsided,  $S^c$ ,  $S$ ,  $s$

The term "colorsided" was probably originated by WRIEDT (1925). The character is found in a number of European breeds of cattle, and it is also found amongst yaks and zebus. In McCANDLISH's paper (1920), dealing with crosses between purebreds and scrubs, there is a photograph of an Arkansas scrub which is colorsided, and also one of her colorsided offspring by a purebred Holstein bull.

The term is to a large extent self-explanatory. Colorsided animals are pigmented on both sides, with the remainder of the body white. Thus, they have a fairly wide white stripe along the entire backbone, and the belly and brisket are white. There is also some white on the head and legs. The line of demarkation between the pigmented areas and the white is very irregular, and some mottling is generally found in the white areas on the head.

The data on the inheritance of colorsided have been collected by WRIEDT (1925). A number of different matings are reported, but the numbers are comparatively small. One exception is the mating of Telemarks and Troenders, colorsided Norwegian breeds, with selfs, such as the Aberdeen-Angus.<sup>†</sup> There were eighteen offspring altogether, and all of them had white bellies and short, narrow, white spinal stripes, but had no white whatever on the heads and necks. WRIEDT concludes that colorsided is incompletely dominant to self.

Coloursided animals were mated to two different recessive white-spotted (*ss*) breeds. The two individuals used in the first, a red-and-white Swedish breed, were males and had only a small amount of white, which was restricted to the belly. The numbers here, also, are fairly large. Fourteen offspring altogether were produced. All had less white than the colorsided parents. WRIEDT concludes that colorsided is incompletely dominant to the white spotting of the Swedish breed. I am inclined to think that the apparent incomplete dominance is due to the plus modifiers (*Lw*, et cetera) carried by that breed. This becomes more apparent when the next cross is considered.

The other breed with recessive white spotting was the Holstein-Friesian. It was crossed with Telemarks and the four offspring were found to have the typical colorsided pattern of the Telemarks. One of the  $F_1$  cows was backcrossed to a Holstein-Friesian bull and produced three daughters. One was colorsided and the other two were typically Holstein in regard to color markings. The colorsided daughter was mated to another Holstein bull and produced a colorsided female offspring, which when mated to a Holstein bull also had a colorsided offspring. WRIEDT concludes that colorsided is completely dominant to the recessive white spotting of Holsteins. None of the Holsteins were described in regard to the amount of white they carried. The chances are, however, that they had more white than the two Swedish red-and-whites described above.

If WRIEDT's statements are taken literally it would mean that, since he considers colorsided dominant to both self and recessive white spotting, he also considers the three factors to be allelomorphic. He does not use any letter to designate the factor for colorsided, thus making it difficult to determine his exact ideas on the subject. His evidence, however, seems to fit in best with the multiple allelomorphic explanation. The alternative explanation would be that colorsided is due to the combined action of two factors, that for self (*S*), and a white spotting factor, epistatic to self, which is responsible for the white found in a colorsided animal. If larger numbers had been obtained in the backcross of  $F_1$  with Holsteins, the question could have been settled with more certainty. In such a cross, if the two factor theory were correct, selfs should have been produced in addition to

the colorsided and Holsteins. Also, on such a theory, one would expect some colorsided animals to occur which showed recessive white spotting. These should be found occasionally also in the colorsided purebreds, since one would not expect all the individuals in the breed to be homozygous for the two factors mentioned above. There is nothing in WRIEDT's paper, however, to indicate that they are ever produced.

Adopting the multiple allelomorphic explanation, it seems advisable to use the symbol  $S^c$  for the colorsided factor. Like its allelomorphs  $S^H$  and  $S^D$ , it is responsible not only for the white but for the pigmented part of the coat. Based on the information supplied by WRIEDT,  $S^c$  is incompletely dominant to  $S$  and completely dominant to  $s$ . There seems to be evidence that the modifying factors which change the expression of  $s$  have corresponding effects on  $S^c$ . It will be of interest to obtain the phenotypic interactions of  $S^H$ ,  $S^D$  and  $S^c$ . One is probably justified in predicting that any heterozygote will show the effects of the two factors that are present.

### *Dominant White Spotting*

#### *Inguinal White, $In$*

The evidence furnished by GOWEN (1918) is fairly convincing that the factor which is responsible for inguinal white spotting is not allelomorphic with  $S$  and  $s$ . If it were, it would not only produce the small white spot in the inguinal region (on either the teats or the udders of females and on either the rudimentary teats or the sheath and scrotum of males), but would also cause the remainder of the body to be pigmented. There are animals, especially in the Aberdeen-Angus breed, which are entirely pigmented except for the inguinal white spot, but there are also Holsteins which when mated to selfs produce offspring with inguinal white spotting, thus demonstrating that  $ss$  animals may carry the inguinal white factor.

GOWEN did not discuss the possibility of the factor for inguinal white being part of an allelomorphic series. He stated that the character is due to a dominant factor.  $In$  is suggested here as the symbol for this factor. Its allelomorph,  $in$ , is merely the absence of the dominant gene.

Inguinal white can be demonstrated best in animals carrying the factor for self, in this way proving that  $In$  is epistatic to  $S$ . Animals that are  $ss$  usually are white along the entire underline, making it impossible to determine by inspection whether or not  $In$  is present. The presence of  $In$  in an  $ss$  animal can be shown only by a breeding test:

$$\begin{array}{lll} \text{White Spotted} & \text{Self} & \text{Inguinal White} \\ ss\ InIn \times SS\ inin & = & Ss\ Inin \end{array}$$

Such a cross would on the face of it appear to be an example of the in-

complete dominance of *S* to *s*. This possibility was referred to earlier when the last named allelomorphs were discussed.

The data presented by GOWEN are the following. The mating together of heterozygous inguinal spotted animals produced 21 with the spot and 6 without. Heterozygous animals mated to recessives had 10 offspring with the spot and 8 without. GOWEN feels somewhat hesitant about fully accepting the factor. It should be quite a simple matter to test its validity by making careful observations within the Aberdeen-Angus breed where recessive white spotting is not present to obscure results.

### *Whitening, w*

The Jersey breed color is very often described as "fawn." On closer inspection one finds that this color is made up of several components, one of which has hitherto not been described. Besides carrying the black spotting factor, *Bs*, and some form of dilution (either *i* or *D*), there is also apparently another factor which produces the effect here given the name of "whitening." The most striking manifestation of this gene is the whitish muzzle which surrounds the usually black nose. The factor, which is recessive and is designated *w*, also causes some of the hairs on the inside of the ear to be white or whitish, and is responsible for many white or whitish hairs on the belly, udder and inside of rear legs. The amount of whitening varies considerably. Often it extends over the entire body, but always to a less degree dorsally than ventrally. In extreme cases the apparent diluting effect brought about by the factor is marked. Animals with extreme whitening, which at the same time carry much black, due to *Bs* and modifiers, are grayish in appearance.

No formal experiments have been conducted to determine the mode of inheritance of whitening. The evidence given here is not absolute, but it all points to the conclusion that the character is due to a recessive factor. Its dominant allelomorph, *W*, prevents the appearance of the character.

Some of the supporting evidence will be mentioned. In the first place, all Jerseys seem to be homozygous for *w* even though no selection apparently has been practiced. Secondly, when Jerseys are crossed with Angus, Holsteins or Herefords, the whitening does not show in the  $F_1$  generation, but does reappear in the  $F_2$ .

There is a possibility that *w*, in the homozygous condition, may have an effect in the presence of *B*. PARLOUR (1913), describing an  $F_2$  animal from a Jersey-Angus cross, states that it is black "with fawn showing through." COLE (1924), describing an  $F_2$  animal from a similar cross, calls it a "very dark type of Jersey." I have seen a purebred Jersey cow that was practically self black except for the whitish hairs on the muzzle. She was mated to a typically colored purebred Jersey bull and produced a daughter simi-

lar to herself. It seems reasonable to assume that mother and daughter, and also the animals mentioned by PARLOUR and COLE, carry *B* instead of the *Bs* factor usually found in Jerseys. If they do carry *B*, it means that *w* has less effect in the presence of *B* than it has in the presence of *Bs*. A well-informed Jersey breeder has made the statement that many of the "Majesty" line of purebred Jerseys are as black as the mother and daughter described above.

COLE (1924), commenting on the  $F_2$  generation of the Jersey-Angus cross, states, "In no case so far, however, has the typical light fawn of the Jersey been exactly reproduced." The reason for this becomes apparent when one realizes that a typical Jersey should be homozygous for at least four recessive genes. Such a statement is true especially if the Angus  $P_1$  carries the factor for brindling (*Br*). The typical Jersey would be of the composition *bb Bs(Bs or bs) brbr ii ww*. The dominant dilution factor, *D*, does not enter into the above cross since the black of the  $F_1$  is practically as intense as that of the Angus  $P_1$ .

#### *Pigmented (Black) Skin Spotting, Ps*

Both the typical Guernsey and the typical Shorthorn are of the composition *bb bsbs*. If one examines the noses of individuals in either of these breeds he will find that they are usually yellowish-brown in color, the intensity of the color of the skin of the nose being correlated to a large extent with the shade of the hair. Since Shorthorns have hair of a deeper red than Guernseys, they also have noses that are more brownish in color. White Shorthorns have as darkly pigmented noses as the reds. The reason for this is that they carry the same modifiers of intensity as the reds, but the presence of these modifiers is not so apparent, due to the epistatic effect of *N* in the homozygous condition. This pigmented condition of the nose is not the result of a separate factor, but is merely the effect of the interaction of red (*R*) with factors normally present in the two breeds.

The noses of all Guernseys and Shorthorns, however, are not entirely yellowish-brown. Many have black spots of varying sizes interspersed through the brown, and sometimes the entire nose is black. In Guernseys one also finds black hairs on the muzzle, which surrounds the nose. The black hairs on the muzzle, however, are not necessarily associated with black spots on the nose. I have not seen any Shorthorns with black hairs on the muzzle. This may be due to the small number which have been examined for that purpose.

In other breeds, like the Holsteins and Ayrshires, the nose may occasionally be entirely devoid of pigment because of the action of the *s* factor in combination with its modifier, *lw*. Noses devoid of pigment would be pinkish in appearance since the blood has an opportunity to show through.



Holsteins and Ayrshires carry either *B* or *Bs*. Both of these factors cause black pigment to form in the nose. Both breeds are *ss*. The combination of either *B* or *Bs* with *s* generally results in an animal with black as well as white spots on the nose. Such black spotting is different from that, described above, which occurs in Guernseys and Shorthorns, although there are indications that the Guernsey type of black spotting also is found in Holsteins and Ayrshires.

Up to the present there have been no published reports on the inheritance of "smutty" (black-spotted) noses in Guernseys and Shorthorns, but there is a published statement by Miss PITT (1920) on the inheritance of the character in Herefords. She asserts that when pigmented nose occurs in the breed, the pigmented spots may be brown alone, black alone, but most frequently they are both black and brown. She does not try to account for the brown spotting. Since Herefords are *bb bsbs* like Guernseys and Shorthorns, it seems reasonable to assume that they would be brown-nosed also were it not for the *S<sup>H</sup>* factor which usually is responsible for the head being devoid of pigment and therefore white. In certain cases, as shown by Miss PITT, some of the brown shows on the nose in spite of the *S<sup>H</sup>* factor. It is not known whether or not this is due to a modifier of *S<sup>H</sup>*.

There seems to be no question but that there is an inherited tendency for black pigment to form in the skin even though the hair above is white. I have seen the character in both Holsteins and Ayrshires. It is easier to detect it in these breeds because there are so many individuals with a large amount of white spotting. Guernseys and Shorthorns have very little white hair, and it is much more difficult to determine the skin color under pigmented hair. The best place, other than the nose, to look for either black or brown skin spots in Guernseys and Shorthorns is the udder and the teats. Oftentimes these spots are found in both the inguinal region and the nose, but occasionally they are found in only one or the other location.

Miss PITT (1920) has postulated a dominant factor for pigmented nose. Her data are very meager—the mating of heterozygotes with recessives resulted in three with pigmented noses and three without. A purported homozygous dominant mated to recessives produced four, all of which had pigment in the nose. She advances no evidence to show that when both parents have clean noses they always breed true.

Under the circumstances it seems more reasonable to suggest a dominant factor *Ps*, for black pigmented skin spots, which may fall almost anywhere on the body, but which would be most noticeable on the bare skin. With such a factor it would be possible for animals to carry it and still have clean noses. It would be very difficult to rid a breed of a factor of this kind if one took the nose only into consideration in determining the presence of the factor. Animals of the composition *psps*, on the above interpretation,

would have no black skin spots whatever, at least none that are due to *Ps*.

There is some evidence, far from complete, which indicates that the *Ps* factor expresses itself gradually in the growing animal. Up to the present it is based entirely on the apparent fact that young animals are more free from the character than older ones.

#### GENETIC COMPOSITION OF VARIOUS BREEDS

The foregoing account includes the description of a large number of characters for which the mode of inheritance is fairly well established. It also includes a few about which there remains some question. It is now proposed to take all of the above genes into consideration in presenting the factorial composition in regard to color for a number of the best known breeds of cattle in the United States. At the same time other characters in each breed will be mentioned whose inheritance has either not as yet been determined, or has not been fully enough investigated. This last list will not by any means be complete.

It will be impossible to take into consideration all the genes described, in giving the factorial composition of each breed, since each breed has not been tested for all of the genes. The method that will be followed is to present the composition as based upon the appearance of the most common type in the breed. If there are any phenotypic variations, the genes for these are placed directly under those representing the common type. The genotypic variations will not be indicated.

Sometimes it is difficult to determine what are the normal characteristics of a breed. For example, there are no data available concerning the frequency of inguinal white (*In*) in either the Angus or the white-spotted (*s*) breeds. I am assuming that this character is usually lacking. Since red (*R*) is assumed always to be present, it will save space by not including it in the factorial composition. Where there are modifying factors these will be placed directly after the genes they modify. The presence of some factors, which have no phenotypic effect in certain combinations, has been determined by means of breeding tests. These genes will be mentioned in the discussion after the genetic formula for the breed has been given.

#### *Aberdeen-Angus*

With the above considerations in mind, the genetic color formula for a typical Aberdeen-Angus may be represented as follows:

$$\begin{array}{c} BB \ dd \ II \ inin \ nn \ SS \ WW \ WnWn \ wpwp. \\ Inin \end{array}$$

There is some variation in shade of black in the Angus breed. Such variation may be due to the fact that a few animals are *ii*, but there is no

evidence on the subject. Besides, much remains to be learned concerning the genetic cause for most of the variation in intensity in all of the breeds.

Some information concerning hypostatic genes carried by the Angus breed may be obtained when one takes into consideration the red calves that are occasionally produced by heterozygous blacks (*Bb*). COLE and JONES (1920) do not give a detailed description of such animals, but one gets the impression from their paper that the reds they have seen are of a uniformly deep shade and therefore do not carry the gene for black spotting, *Bs*. WALLACE (1907) has photographs of two red Angus which give every indication of being of the composition *bsbs*. All the evidence available fits in with the assumption that the Angus breed as a whole is homozygous for *bs*. On the other hand, evidence from the  $F_2$  of Jersey-Angus crosses (COLE 1925) demonstrates that individuals of the Angus breed may carry the gene for brindling, *Br*.

Certain other variations have been observed. TEMPLETON (1923) reports on a white-faced red calf, produced when a *Bb* Angus bull was mated to a Hereford cow, that had "a dark muzzle and a dark tongue." Presumably the muzzle and tongue carried black pigment, and the hair did not. The black pigment probably would not be due to *Ps* since it was not in spots. There seems to be a possibility here of a hitherto unreported gene that causes all of the skin to carry black pigment even though the hair is red or white.

At the International Livestock Exposition of 1931, held at Chicago, Professor A. D. WEBER of the KANSAS STATE COLLEGE took special note of the red Angus shown, and has kindly given me permission to make use of the information he obtained. One animal is of especial interest. It was a purebred red steer and had the typical brownish nose and eyelids, but in addition had distinctly black hairs on the face and the poll. If it had carried the *Bs* gene the nose and eyelids would have been black. A possibility, pointed out by Mr. E. N. WENTWORTH, is that the apparently black hairs actually may be very intense red. He has examined under the microscope "black" hairs from red Shorthorns and has found them to be made up of very closely packed red granules.

One other point might be mentioned, and that is in connection with the presence or absence of the gene for pigmented leg (*Pl*) in the Angus breed. Invariably, so far as I have been able to ascertain, an animal, usually black, with a clean white face is produced when a purebred Angus is mated to a purebred Hereford. All purebred Herefords are *plpl*, and the crossbred sometimes would be "brockle"-faced if *Pl* were ever carried by the Angus parent. Thus we are justified in concluding that at least most Angus are of the composition *plpl*.

*Holstein-Friesian*

From a color gene standpoint Holsteins differ from the Angus breed chiefly in that they carry recessive white spotting, *s*, while the Angus are *SS*. Since the Holsteins are *ss* it makes it possible to determine what modifiers of *s* they carry, but makes it impossible to ascertain by inspection whether or not they carry *In*. The genetic composition of typical Holsteins therefore would be:

$$\begin{array}{cccccccc} BB & dd & II & nn & ps\ ps & ss & Lwlw & plpl & WW & WnWn & wpwp \\ & & & & Ps\ ps & & lwlw & & & & \\ & & & & & & LwLw & & & & \end{array}$$

It is assumed that animals with an intermediate amount of white spotting (*Lwlw*) are most common and that those with a very small amount (*LwLw*) are least common.

DETLEFSEN (1920) reports a peculiar case, from the standpoint of inheritance, in Holsteins, the information concerning which was supplied by the owner of the herd in which it occurred. Two albinos (even the eyes were devoid of pigment, and therefore pink) of opposite sex were produced from normally pigmented parents. These albinos when mated produced four albino offspring. From the evidence at this point the albinism would seem to be due to a recessive gene. However, when the albino male was mated to grade Holstein cows, all of the twenty offspring were albinos, the male thus appearing to be a homozygous dominant. It seems possible that the owner was not so anxious to get at the facts as to prove the "prepotency" of his albino herd, for that reason making the albinism due to a dominant as well as to a recessive gene when it probably was due to only the latter.

An animal described by LAUPRECHT (1928) possibly carries a new gene. It is a tricolor produced by normally pigmented Holsteins, and is unique in that the black spots are sharply differentiated from the red. In the colored plate it looks distinctly different from a *Bs* animal, such as an Ayrshire, where the black tends to blend with the red. One gets the impression that the animal carries *B*, but that this is only partially extended as in tortoiseshell guinea pigs. The cow showing the above character unfortunately proved to be sterile.

Four common variations might be referred to at this time. One is the tendency, previously mentioned in this paper, for animals to be produced with one or more legs entirely pigmented. It is not known whether or not this has an hereditary basis. Another variation is the "Ayrshire" type of spotting. In most Holsteins the white spotting is so distributed that the black areas are in big blotches, while in Ayrshires the pigmented areas form small spots. I have seen a calf, produced by an Ayrshire cow mated

to a Holstein bull, which had typical Holstein color markings. Such a result would be in line with the interpretation that Holsteins in general carry a dominant modifier of *s*, while the Ayrshires carry the recessive allelomorph. Additional data could be obtained within the Holstein and the Ayrshire breeds as well as by breed crosses.

A third variation also has to do with white spotting. I am referring to animals having heads almost entirely white even though the remainder of the body may be to a large extent pigmented. As previously stated, either a dominant modifier of *s* or a dominant gene for white spotting is responsible.

The last variation is evident only in calves. Animals possessing it have hair which fades to a reddish slate color previous to shedding. In other calves of the same age there will be no such change, the hair remaining black throughout. The character is probably inherited, but the data available are very incomplete.

### *Jersey*

The chief difference between a Jersey and either an Angus or a Holstein is that the two latter carry *B* while most Jerseys are *bb*. Jerseys also are distinctive in that they are *ww*. The formula for typical animals of the breed is:

$$\begin{array}{ccccccc}
 bb & brbr & BsBs & ML & dd & ii & nn & SS & & ww & Wn & Wn & wpwp \\
 Bb & & LL & Dd & & ss & Lw & Lw & & & & & \\
 & & & & & & MM & & & & & & \\
 & & & & & & bsbs & & & & & & 
 \end{array}$$

The above formula should convey to the reader a number of different points, all of which have previously been made in the body of the paper. Two might be mentioned here. In the first place the extremely black animals with white muzzles, occasionally found, are assumed to be *Bb*, and, in the second place, those with flesh colored noses, *bb bsbs*.

Some explanation is necessary in regard to the placing of *dd* as the most usual composition of Jerseys and of *Dd* as being of rarer occurrence. In the previous discussion of *D* and *i* it was stated that *D* had a more pronounced diluting effect than *i*. Black animals carrying *D* are duns. However, most Jersey-Angus crossbreds are intense blacks, thus indicating that the Jersey parents were *dd*. KUHLMAN (1915a) states that duns are sometimes produced when the above cross is made. One would have to assume that the Jersey parents of these animals carried *D*. It is not known with certainty whether a Jersey carrying *D* can be distinguished from a *dd* animal, but, since *D* has a more pronounced effect than *i*, one can be reasonably certain that cream colored animals have a greater probability of carrying *D* than those of a darker shade.

*Ayrshire*

Ayrshires differ from Jerseys in two main particulars. They are white-spotted, as contrasted with self, and are intense rather than dilute. Their composition is:

*bb brbr BsBs ML dd II nn psps ss lwlw plpl WW WnWn wpwp*  
*Bb Brbr LL Psps Lwlw Plpl*  
*MM LwLw*  
*bsbs*

The formula shows that there are a number of alternative conditions in the breed. The vast majority, if not all, of the animals in this country are *bb*, but there are some in Scotland that carry *B*. A few in this country show brindling (*Br*), and there are also a few that have flesh colored noses and are at the same time without any black pigment in the hair (*bsbs*). Some show black pigment in skin which is under white hair (*Ps*), while a larger proportion have pigmented areas of hair above the hoofs and below the knees (*Pl*).

There are a number of peculiarities in regard to white spotting in Ayrshires which may be due to modifiers of *s*. One has already been pointed out in the discussion of Holsteins. I refer to the tendency for the pigmented spots to be small and therefore comparatively numerous. If one examines a number of animals he will find considerable variation in the degree to which this breaking up into small pigmented areas has been carried on. An apparently accompanying phenomenon is the irregularity in outline of both large and small spots.

Another type of small spots entirely different from the above also occurs. That already described seems to be due to the breaking up of a large pigmented area into numerous smaller ones. The type about to be described occurs in white areas and consists of small pigmented flecks. This kind of spotting would tend to increase the amount of pigment carried by the animal while the other would tend to decrease it.

Still another peculiarity in regard to white spotting in Ayrshires is the presence of "halos." These surround the pigmented spots and consist of either black or red hairs, or both, that are intermingled with the white, and extend out only a short distance.

*Hereford*

The genetic color formula of the typical Hereford is rather long because of the special modifiers of the Hereford pattern:

*bb bsbs dd II nn psps S<sup>#</sup>S<sup>#</sup> rnrrn rere LwLw plpl WW WnWn wpwp*  
*Psps Rnrrn Rere Lwlw*  
*Bs? lwlw*

Miss PRIT (1920) believes that yellow Herefords carry a dominant dilution factor, but there seems to be no question that it is not *D*. Evidence from crosses shows that many Herefords carry the brindling gene, *Br*. This, however, is not apparent phenotypically in purebred animals. The fact that some animals have what seem to be black hairs in spots fits in with the supposition that *Br*s may sometimes be carried. Further evidence is necessary before the question can be settled. As pointed out previously, more data are necessary in order to work out the inheritance of "lineback," apparently due to a recessive gene.

### *Shorthorn*

The most characteristic color gene in Shorthorns is *N*, which is responsible for roans (*Nn*) and whites (*NN*). The breed is also noteworthy in that, although no animal seems to carry the gene for self (*S*), a large majority of the *nn* individuals show very little white spotting. Some of the white spotting on roan animals apparently is due to *N* and its modifiers. Shorthorn breeders have made a conscious effort to reduce the amount of white spotting in their animals. They were not aware of the genes involved, but they have unconsciously retained *Pl* in order to keep pigment on the legs and head, they have decreased the amount of white by making most of their animals *LwLw*, and, finally, they have kept a third dominant modifier (tentatively assumed to be *Rn*) which made possible a further reduction. The formula for the breed is:

$$\begin{array}{cccccccccccccccc}
 bb & bsbs & dd & II & Nn & RmRm & psps & ss & LwLw & RnRn? & PlPl & WW & WnWn & wpwp \\
 & & & & & rmrn & Psps & & Lwlw & rn rn & plpl \\
 & & & & nn & & & & lwlw \\
 & & & & NN & & & & & & & & & 
 \end{array}$$

There are probably many unsettled questions in regard to color inheritance in Shorthorns. Only two will be mentioned here. In the first place, there seems to be no doubt but that there are a number of modifiers affecting the expression of roan. One, *rm*, has already been postulated in this paper. The others, judging by the kind of variations found in the Shorthorn breed, apparently control the relative numbers of white and pigmented hairs, and still others control the uniformity with which this relationship extends over the entire body. There is also possibly an age effect, some modifiers tending to increase the relative numbers of white hairs with increasing age, and others having the opposite effect.

The second problem can be stated more briefly. I am referring to the extreme intensity of red found in the breed. Shorthorns are a deeper red than any other of the breeds considered in this study, and there is undoubtedly a factorial basis for this difference.

*Guernsey*

The typical Guernsey has the following color formula:

*bb bsbs dd ii nn psps ss LwLw plpl WW WnWn wpwp*  
*Dd? Psps Lwlw*  
*lwlw*

There is probably some question as to whether or not the Guernsey breed ever carries dominant dilution (*D*). It depends partially on whether the "brownish-black" obtained by GOWEN (1918) in an Angus-Guernsey cross-bred was a true dun. There is the possibility that the Angus parent was *Ii* and that the crossbred was a recessive dilute (*ii*).

Many Guernseys have hair which is much more dilute on the muzzle and on the inner sides of the rear legs than on the remainder of the body. It is not improbable that this is due to the same gene (*w*) that causes whitening in Jerseys. If it is, we would have to assume that the effect is less pronounced in Guernseys than in Jerseys, possibly due to the absence of the *Bs* gene in the former breed. The matter could be tested by an examination of *bsbs* purebred Jerseys or by crossing a Jersey with a light muzzled Guernsey.

Occasionally what appear to be black hairs are found interspersed with the red on various parts of the body of purebred Guernseys. The area covered is comparatively small, as in the red Angus previously described, and usually consists of the muzzle or the tail. The skin of the nose of such animals may or may not carry black pigment. If these effects are due to the *Bs* gene, one would have to assume that they have been greatly reduced because of the selection of minus modifiers.

It has been mentioned previously that a fairly large proportion of Guernseys have small pigmented areas of hair directly above one or more of the hoofs. The pigmented leg gene, *Pl*, does not seem to be the cause for these spots, nor has it been ascertained that any particular gene is responsible. There is the possibility that such spots may occur in *ss* animals that are at the same time *LwLw*, and who therefore have a comparatively small amount of white spotting. If such is the case, *LwLw* Holsteins should also show this type of spotting. Further investigation is necessary.

Two peculiarities that Guernseys have in common with Ayrshires might be mentioned. One is the "halo," the chief difference between the two breeds being that it consists only of red hairs in the Guernsey, while both black and red hairs may be found in it in the Ayrshire, due to the *Bs* gene. The other is the irregularity of outline of the pigmented areas in certain animals. In Ayrshires nearly all individuals have this irregularity of outline, but in Guernseys it is less common. It should therefore be less difficult



to get crucial data on the mode of inheritance in the latter breed since both types of spotting are available for study.

There are several other breeds in the United States that have a fairly large number of representatives, as for instance the Brown Swiss, but I have not had enough personal experience with them to make a critical discussion. The Guernseys, therefore, will be the last breed to be considered.

#### SOMATIC COLOR MUTATIONS

Several color variations have been reported which without much doubt are somatic mutations. They have not been described as such in the original papers, but they will be given that classification here.

Two cases are from LAUPRECHT (1928), one from HORLACHER (1928), and the fourth is from unpublished material. One of the animals described by LAUPRECHT was a "Schlesischen Landvieh." Individuals in this breed are normally red-and-white. The exceptional animal had in addition to the red-and-white a large patch of black on the left shoulder. From a genetic standpoint one would have to suppose that the animal was *bb*, but that a mutation had occurred in one or more of the somatic cells, and persisted in their descendants, changing a *b* to *B*.

HORLACHER's case is similar to the above, and the explanation would be the same. Here a black spot (17 cm X 8 cm) enclosed by red was found on the left side of the neck of a purebred Hereford. HORLACHER states that black spots such as that described are occasionally found in both purebred Herefords and Shorthorns.

LAUPRECHT's second case was found in a Holstein-Friesian herd in Holland. The animal was a black-and-white with a small red spot about 5 cm across. Red-and-white Holsteins are fairly common in Holland and the females can be registered. Red-and-white males, however, cannot. In the United States no red-and-white Holsteins can be registered. One would therefore expect that a large proportion of black-and-whites in Holland are *Bb*. If the animal described by LAUPRECHT were of this composition the red spot could be explained by assuming that the *B* mutated to *b* in possibly one somatic cell, and that the spot was produced by the descendants of this cell.

The last case to be described was found amongst a large number of Hereford steers on the ranch of Mr. DAN CASEMENT of Manhattan, Kansas. The animal in question was not a purebred, but nevertheless both parents had the typical Hereford color and pattern. The somatic mutation (or mutations) was responsible for the production of two distinct roan areas on the posterior part of the animal. One of these areas, circular in shape and at least one foot in diameter, was on the left thigh, while the other, consisting of two spots, was on the inner sides of both hind legs.

Mr. CASEMENT informed me that amongst his Herefords he has seen black spots similar to the one reported by HORLACHER on one in every three hundred or so individuals, but that he has never before seen an animal with roan areas. Herefords are *nn* in composition. The somatic mutation consisted in the changing of an *n* to *N* in possibly two somewhat separated somatic cells.

Mr. E. N. WENTWORTH states that when the skin of either red or black-haired animals is blistered as a result of the application of concentrated dips or crude oil, or if the branding operation has been incompletely performed, there will subsequently be a sprinkling of white hairs in those areas, thereby producing a roan effect. Mr. CASEMENT's roan-spotted Hereford, however, has not received any of the above types of treatment.

#### SUMMARY

In summarizing the results of a paper of this kind it would be a tedious and at the same time an unprofitable task to attempt to delimit the new material in it. It should be sufficient to state that a complete revision of color inheritance concepts in cattle has been aimed at, and that during this process many changes have been advocated and much new matter added. In presenting the latter it has been deemed inexpedient to give the supporting evidence in detail.

One gene, that for red (*R*), is assumed to be always homozygous. Seventeen factor pairs and an allelomorphic series made up of five genes are described in detail and their interactions, as far as they are known, given.

The factorial composition, from the standpoint of color, is given for the seven leading cattle breeds of the United States. Attention is also called to a number of other color characters in these breeds of which the mode of inheritance has not as yet been sufficiently determined. Several somatic color mutations are described and an attempt is made to explain their occurrence.

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# INHERITANCE OF VENTRAL SPOTTING IN MICE

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The two best known types of white spotting in mice are "black-eyed white" and "piebald". The former, *Ww*, is dominant (lethal in a homozygous condition) while the latter, *ss*, is recessive to self. A possible third distinct form, "all white" (*whwh*) has been mentioned by KEELER (1931). In addition, a number of other types, at least some of which are the result of modifying genes on basic piebald, have been noted in the literature.

DUNN (1920, 1925) described two modifications of the piebald pattern. In one, which he designated as "white face spotting", the white areas were restricted to the face; and in the other, "belt", to a band around the body. By inbreeding, he succeeded in fixing the white face pattern but was less successful with belt.

LITTLE (1917, 1926) reported a form of spotting, "blaze", in which the animals showed a small, white forehead spot. Besides the main gene responsible for this condition, he concluded that there were several modifying genes influencing the amount of white.

The same author (1924) observed two localized forms of spotting, "white tail tip" and an unpigmented band around the tail, "white on tail". In the race in which these types were observed, he reported that occasionally the tail spotting was transferred to the belly, such animals behaving in inheritance like phenotypically tail-spotted mice.

Ventral spotting appears to be of rather frequent occurrence in mice. DUNN (1920) found its appearance dependent on a modifying gene in conjunction with piebald in the heterozygous condition (*Ss*). ALLEN (1914) considered a mid-ventral patch generally to be the first unpigmented area developing in mammals.

In one inbred strain in our laboratory more than 50 percent of all individuals show a white ventral patch. The mice of this strain (C-57 Brown) are intense brown non-agouti (*DDbbaa*) in color and directly descended, by brother-to-sister matings with some backcrosses, from two control animals of one of Dr. C. C. LITTLE's previous experiments. The mice reported on in this paper comprise undepleted litters of the thirteenth to the twenty-eighth filial generations, inclusive.

The patches vary in size from 0 to approximately 10 percent of the ventral surface. In the few mice of the highest grades the unpigmented area sometimes extends up onto the side of the animal. All determinations

of grade were made by the senior author, thus reducing the personal error incident to more than one observer, while the junior author is responsible for the computations and analysis. Some animals exhibited "white on tail" or "white tail tip" with or without ventral spotting. It seems probable, however, that in the strain of browns the tail markings and ventral spotting are not conditioned by the same factors. The C-57 Blacks in which LITTLE described tail spotting still produce a majority with such markings while belly spotting is now almost non-existent. The C-57 Browns, with a common origin, show both tail and ventral spotting. Moreover, satisfactory records as to the degree of tail spotting manifested by individual mice were not available so it was deemed advisable to consider only ventral white.

The distribution of the 2166 mice of undepleted litters by grade of ventral spotting is shown in table 1.

TABLE 1  
*Distribution of offspring in brown stock grade of ventral spotting.*

	0	1	2	3	4	5	6	7	8	9	10	TOTAL
Sexes combined	984	658	260	92	80	46	13	9	15	4	5	2166
Percent	45.4	30.4	12.0	4.2	3.7	2.1	0.6	0.4	0.7	0.2	0.2	
Males	420	340	154	60	55	25	8	3	10	3	3	1081
Percent	38.6	31.5	14.2	5.6	5.1	2.3	0.7	0.3	0.9	0.3	0.3	
Females	563	319	106	32	25	21	5	6	5	1	2	1085
Percent	51.9	29.4	9.8	2.9	2.3	1.9	0.5	0.6	0.5	0.1	0.2	

From this table it is evident that not only are more males than females spotted but also that the grade of the former tends to be higher.

Table 2 gives distributions and mean grades of offspring from matings differing in degree of ventral spotting.

TABLE 2  
*Distribution of offspring in different types of matings.*

MATING		0	1	2	GRADE OF VENTRAL SPOTTING						10	MEAN
					3	4	5	6	7	8		
♀ Self × ♂ self	♂	130	82	36	7	5	2	1	1	1		.85
	♀	173	82	17	5	4	4		1	1		.63
♀ Grade 1 × ♂ Grade 1	♂	38	37	15	11	10	5	2		1	1	1.63
	♀	65	30	14	2	2	4	1	3		1	1.06
♀ Grade 2-7 × ♂ Grade 2-10	♂	22	23	9	7	6	6		2	5	1	2.22
	♀	27	26	6	6	5	6	1			1	1.56

It seems clear that a distinct correlation is present between grades of spotting in parents and offspring. This correlation, however, is imperfect since self parents produce offspring with a high grade of ventral spotting while spotted parents give birth to self animals.

The great majority of mice in the strain of browns are of three lines, A, C and CD. If the degree of ventral spotting is influenced by genetic factors, it might be expected that the lines would exhibit differences in the presence and extent of unpigmented areas. The distribution of ventral spotting in the three lines is listed in table 3.

TABLE 3  
*Distribution of ventral spotting in the three chief lines.*

		GRADE OF VENTRAL SPOTTING											MEAN
		0	1	2	3	4	5	6	7	8	9	10	
Line A	♂	32	22	15	2	6	2			1			1.25
	♀	40	27	10	7	2	2		1				1.04
Line C	♂	122	102	37	8	9	6	3	1				1.01
	♀	178	96	26	2	9	5		3	2			.79
Line CD	♂	251	181	92	47	30	15	4	2	8	3	3	1.35
	♀	318	181	65	26	13	13	5	2	2	1	2	.95

The anticipated differentiation in the lines has apparently taken place since the mean grade is significantly lower, at least for the males, in Line C than in Line CD. Line A appears to be similar to CD although the numbers are few.

Since tables 2 and 3 make it seem probable that the presence and degree of ventral spotting are in part at least determined by genetic factors, parent-offspring correlation tables were constructed in order to show more exactly the extent to which variation in parental grade influenced variation in the grade of the offspring. The correlation between mid-parental grade and grade of offspring was  $+0.258 \pm .014$ . The square of this figure would indicate that slightly over six percent of the variation among the offspring was determined by variation among the parents.

The question which next suggests itself is the relative degree to which sire and dam influence spotting in the offspring. These correlations are given below:

Grade of spotting of sire—Grade of offspring  $r = +0.269 \pm .013$

Grade of spotting of dam—Grade of offspring  $r = +0.145 \pm .014$

These figures show that the correlation is positive and significant in both cases, but markedly higher with the sire than with the dam. A truer picture of the relative effects of the two parents may be given by the use of partial

correlations. This discloses that the correlation between sire and offspring with dam held constant is  $+0.243$ ; that between dam and offspring with sire held constant is  $+0.086$ . The difference in the apparent influence of the two parents is thus further augmented.

The coefficients of correlation above have not taken into consideration the sex of the offspring. This factor is dealt with in the figures following:

	r	r (partial correlation)
Grade of spotting of sire		
—Grade of male offspring	$+0.300 \pm .019$	$+0.268$ (Dam held constant)
Grade of spotting of dam		
—Grade of male offspring	$+0.168 \pm .020$	$+0.093$ (Sire held constant)
Grade of spotting of sire		
—Grade of female offspring	$+0.226 \pm .019$	$+0.208$ (Dam held constant)
Grade of spotting of dam		
—Grade of female offspring	$+0.118 \pm .020$	$+0.077$ (Sire held constant)

Both sire and dam show slightly higher correlations with male than with female offspring although the differences probably lack significance.

It appears from the available evidence that heredity accounts for only a small portion of the variation in degree of ventral spotting in our strain of mice. The possibility that ventral spotting is due to either a single dominant or recessive gene is at once precluded. If dominant, self animals could not throw spotted offspring while if it were recessive, self young could not be produced by spotted parents. Of course if normal overlaps were postulated, these objections might be vitiated. That our ventrally spotted mice, like DUNN's, are heterozygous piebalds is likewise precluded, since matings involving such parents do not produce the requisite piebald young. A number of genes may be involved although it might be expected that practical homozygosity would have been attained after 13 generations of inbreeding. The reason for the greater influence of the male in determining the grade of spotting of the offspring is not at all clear.

Since heredity—as measured by the square of the parent-offspring coefficient of correlation—accounts for but little more than six percent of the variance, non-genetic factors must play the major part in determining the degree of ventral spotting. The data permit an analysis of several such possibilities.

WRIGHT (1926) found that the age of the dam was of more importance than heredity in determining the grade of spotting in an inbred race of guinea pigs. In our material, there was present a correlation of  $+0.105 \pm .014$  between age of dam (in almost all cases the sire was of the same age) and grade of ventral spotting. Although significant, this is very low and would indicate that age accounts for about one percent of the variation in amount

of white in the offspring. Birth rank (position of litter in the series) likewise proves of slight importance as a factor with  $r$  equalling  $+0.068$ . In this case only males, both parents of which were self, were included. Since birth rank is largely a function of age it was to be expected that similar results would be encountered.

Still another possibility is that season of the year is a factor in influencing ventral spotting. Table 4, comprising offspring of self  $\times$  self matings, affords scant evidence of this.

TABLE 4  
*Time of year and ventral spotting.*

SEASON OF BIRTH		GRADE OF VENTRAL SPOTTING								TOTAL	MEAN
		0	1	2	3	4	5	6	7		
Dec. Jan. Feb.	♂	47	19	6	1	1	2			76	.63
	♀	45	20	5	1		2			73	.59
Mar. Apr. May	♂	29	27	8	2	1			1	68	.90
	♀	40	27	3		2	1		1	74	.73
June July Aug.	♂	17	8	4	2					31	.71
	♀	24	15	3	1	1	1			45	.73
Sept. Oct. Nov.	♂	37	28	18	2	3		1	1	90	1.06
	♀	64	20	6	3	1			1	95	.55

Although marked fluctuations occur, they are inconsistent and the differences are not certainly significant. Males born in the three autumn months, for example, show the highest average grade, while females born during the same three months display the lowest. Hence, it is probable that season of birth has little if any effect on grades of spotting.

#### SUMMARY

In an inbred strain of mice a white ventral patch ranging in size up to 10 percent of the ventral surface is present in a majority of the animals. These patches are not only more frequent in males than in females but average larger as well.

The presence of white ventral spotting seems to be due neither to a single dominant nor recessive gene. The occurrence and extent of these markings, however, do appear to have an hereditary basis. In this strain of mice, the three chief lines show a tendency towards differentiation in the amount of spotting present. The coefficient of correlation between mid-parental grade and grade of offspring is  $+0.258$ . When the method of partial correlation is employed, the value of  $r$  for grade of sire and grade of offspring (with dam held constant) is  $+0.243$ ; that for grade of dam and grade of offspring (with sire held constant),  $+0.086$ . From these figures, it ap-



pears that the sire has more influence on the grade of offspring than does the dam. The total effect of heredity is relatively slight, most of the variation being non-genetic in origin.

The age of dam or sire apparently has a very slight influence in determining spotting since the coefficient of correlation is but  $+0.105$ . Birth rank and season of year likewise have little influence; so most of the non-genetic variation, whether it be due to accidents of development or to other causes, is still unaccounted for.

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# INHERITANCE IN *NICOTIANA TABACUM*. XIII. THE CYTOGENETICS OF "DEFORMED," AN X-RAY DERIVATIVE<sup>1</sup>

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At the present time attention is being directed to the genetics and cytology of conditions arising from spontaneously occurring and induced mosaicism. Where the situation is relatively simple, correspondence of cytological and genetic evidence is obtainable (MOHR 1932). In other cases greater or lesser complexity is involved and the correspondence may not be clearly shown (STEIN 1930, 1932). The complexity in the case under discussion here arises primarily from the fact that we are dealing with an X-ray induced physical attachment of chromosomes. This results in somatic elimination of the products of attachment and also in a series of breakages of the physical union which are reflected in the production of numerous by-products. A consistent cytogenetic interpretation of the situation involved is possible primarily because the "fluted" chromosome, which has already been studied genetically, is involved.

An earlier paper in this series (GOODSPEED 1930) dealt with some products of X-ray and radium treatment of sex cells of *Nicotiana tabacum* var. *purpurea*, which behaved genetically like transgenations. In recent reports (GOODSPEED 1931), the occurrence of at least one of these induced character changes is reinterpreted in the light of additional cytological evidence, as the result of quantitative rather than qualitative alteration. A résumé has recently been given (GOODSPEED 1932) of the results of the various types of cytogenetic investigations under way in the UNIVERSITY OF CALIFORNIA BOTANICAL GARDEN on the categories of alteration in the germ-plasm of *N. tabacum* induced by high frequency radiation. Reference is there made to a morphological type called "deformed," which appeared in  $X_1$  and has been the subject of extended genetic and cytological studies, the results of which are herein reported. The designations  $X_1$ ,  $X_2$ ,  $X_3$ , etc., and  $R_1$  (STEIN 1932),  $R_2$ ,  $R_3$  et cetera, have been used throughout these investigations to signify the successive generations derived from X-ray or radium treatment of reproductive or meristematic tissues. Thus, for example, an  $X_1$  plant is one produced from an X-rayed seed, or from an X-rayed growing point of a seedling, or from a zygote produced by the

<sup>1</sup> The investigations reported on here have been aided by grants from the Board of Research of the UNIVERSITY OF CALIFORNIA, and from the Committee on the Effects of Radiation of the NATIONAL RESEARCH COUNCIL.

union of sex cells one or both of which have been subjected to X-radiation.

Appropriate X-ray treatment of EMC and PMC (PMC = pollen mother cells; EMC = embryo-sac mother cells) of *tabacum* in active meiotic stages is always productive of a high degree of variation in expression of all vegetative and floral characters in  $X_1$  (GOODSPEED 1929). This extended range of alteration in expression is to be seen not only in the immediate progenies but also in subsequent generations following treatment. Variation in form, size, and color of flowers and leaves occurs along with variation in height, habit, and time of flowering, et cetera, but only rarely are deformities due

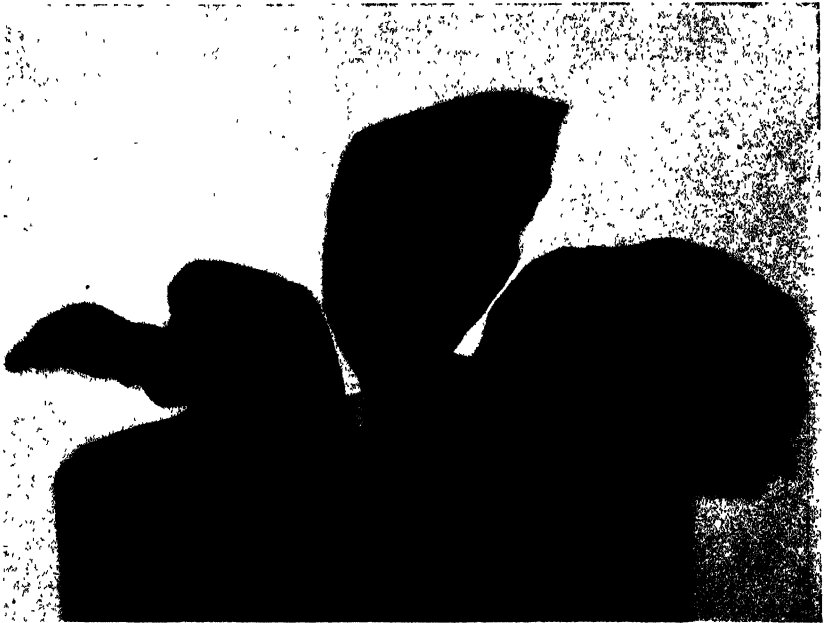


FIGURE 1.—Young *deformed* plant,  $X_1$  (31.212P55), showing abnormalities in tissue development, similar to those seen in the original *deformed* (28.303P51).

to tissue degeneration to be observed. In the case under discussion such a condition is involved. The original  $X_1$  plant (28.303P51) was the result of using normal pollen on a flower which had been subjected to X-radiation (GOODSPEED 1929, for treatment) when it was almost at anthesis. At this stage the EMC are in the meiotic condition. None of the other plants resulting from this cross were as abnormal in appearance as was 28.303P51. From the seedling stage (figures 1 and 6) it was peculiar structurally, with imperfect, asymmetric venation, which made the leaves curl or sometimes allowed them to develop on one side only of the midrib (figure 2). The flowers were flecked with white and much dissected, making the limb very irregular in size and shape (figures 3 and 7, e, f). The anthers



FIGURE 2—*a*, Leaf from control, *N. tabacum* var. *purpurea*; *b*, Five leaf types from an  $X_2$  deformed (29.137P51).

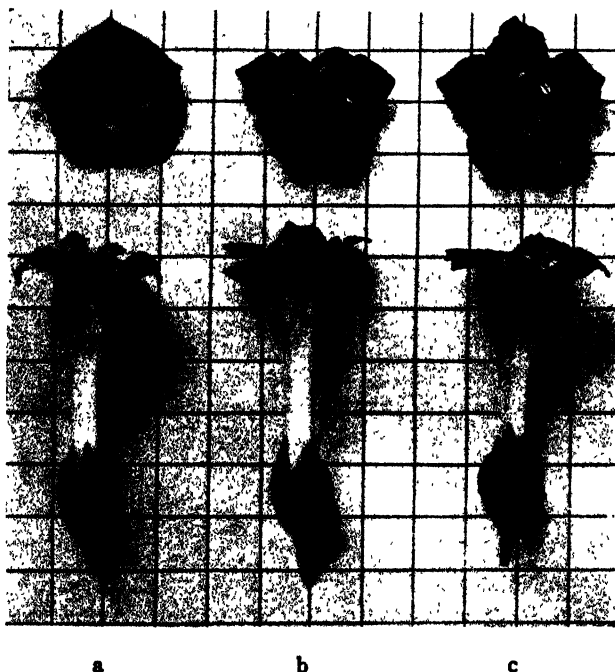


FIGURE 3—*a*, Flower from control, *N. tabacum* var. *purpurea*; *b* and *c*, Flowers from  $X_1$  deformed, showing the white-flecked and dissected limb characteristic of this type.

were of very different types and sizes but contained some viable pollen and dehiscence normally. Some seed was set but often buds were abscised at a very young stage of development. The flower was approximately equal to control in length (55 mm) when best developed but was only about 25 mm in width of limb. The following terminology is here employed in referring to major distinctions in phenotypic expression:

*control* (*cn*) refers to *Nicotiana tabacum* var. *purpurea*, grown in the pure line (figures 2, a and 7, a);

*normal* (*n*) refers to plants in lines derived from *deformed* phenotypically equal to *control*;

*deformed* (*d*), a type characterized by abnormalities (X-ray induced) in tissue production and alignment, the cytogenetics of which is here described (figures 1; 2, b; 3; 5; 6; 7, e, f);

*fluted* (*f*), a distinct character complex elsewhere described (CLAUSEN and GOODSPEED 1926) and involving reduction in flower size, fluting of corolla limb, and other morphological distinctions from *control* (figure 7, b);

*mammoth* (*m*), a type characterized by increase in height accompanied by shortening of internodes and delayed time of blooming (figure 5);

*long-flowers* (*l*) refers to a series of types possessing distinctly increased length of flower. This designation is used to distinguish these derivatives from types already described (CLAUSEN 1931, GOODSPEED 1932) which possess increased flower-size (figure 7, c, d).

The character of the tissue abnormalities peculiar to *deformed* suggests, at first glance, the effects of some such disease as "mosaic" (figures 1 and 2, b). On the other hand, the cytogenetic evidence shows that a diseased condition is not involved and that *deformed* is transmitted through a chromosomal mechanism in which elimination, in mitosis, of chromosomes or parts of chromosomes is concerned. In addition, inoculation tests using unfiltered crude extract from *deformed* plants as the inoculum and vigor-

TABLE 1  
Derivation of progenies from *deformed*.

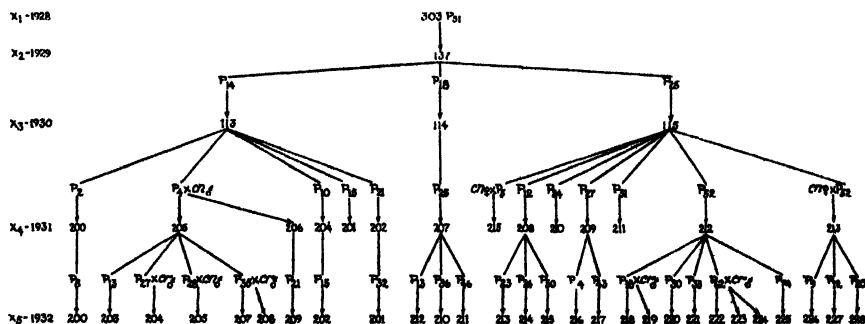


TABLE 2\*  
*Classification of progenies from deformed.*

POPULATION	PARENTAL PHENOTYPE	n	d	f	l	ad	m	df	dl	dm	fm	lm	cf	CCT		NEW TYPES	NEW %	TOTAL			
														n	l			TOTAL	d	f	l
29.137	d	6	30	5	7	..	2	..	..	..	..	..	..	..	..	..	..	50	30	5	7
30.113	d	6	16	4	2	..	..	1	2	..	..	..	..	..	..	..	..	31	19	5	4
30.114	f	11	..	37	..	..	..	..	..	..	..	..	..	..	..	..	1	49	..	37	..
30.115	d	4	17	15	9	..	..	..	4	1	..	1	..	..	..	..	..	51	22	15	14
31.200	f	26	..	22	..	..	..	..	..	..	..	..	..	..	..	..	2	50	..	22	..
31.201	df	..	..	..	..	..	16 <sup>a</sup>	..	..	19 <sup>b</sup>	11	4	..	..	..	..	..	50	19	..	..
31.202	l	3	2	11	14	7	2	..	3	..	..	3	..	..	..	..	..	45	5	11	27
31.204	n	46	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	48	..	..	..
31.205	d	5	13	9	3	..	1	..	..	2	1	1	..	..	..	..	..	35	15	10	4
31.206	d q × cn σ <sup>2</sup>	11	..	8	3	..	..	..	..	..	..	..	..	..	..	..	2	24	..	8	3
31.207	f	30	..	18	..	..	..	..	..	..	..	..	..	..	..	..	..	48	..	18	..
31.208	l	16	..	2	26	4	1	..	1	..	..	..	..	..	..	..	..	50	1	2	31
31.209	dl	17	..	12	20	..	..	..	..	..	..	..	..	..	..	..	..	49	..	12	20
31.210	f	10	..	43	..	..	..	..	..	..	..	..	..	..	..	..	2	55	..	43	..
31.211	dl	46	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4	50	..	..	..
31.212	d	6	25	14	11	..	4	..	3	..	2	4	..	..	..	..	..	69	28	16	18
31.213	cn q × d σ <sup>2</sup>	2	..	1	19	2	..	..	..	..	..	..	..	..	..	..	1	25	..	1	21
31.215	cn q × d σ <sup>2</sup>	12	..	3	8	..	..	..	..	..	..	..	..	..	..	..	3	26	..	3	8
32.200	cn σ <sup>2</sup>	12	..	3	1	..	..	1	..	..	12 <sup>c</sup>	..	..	..	..	15 <sup>d</sup>	6	50	..	..	..
32.201	dl	18	..	..	..	..	..	..	..	..	..	..	..	..	..	10 <sup>e</sup>	3	31	..	..	..
32.202	n	47	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3	50	..	..	..
32.203	l	23	..	..	16	10	..	..	..	..	..	..	..	..	..	..	..	49	..	..	26
32.204	d q × cn σ <sup>2</sup>	30	..	..	1	..	..	..	..	..	..	..	3	14	1	..	..	49	..	3	2
32.205	d q × cn σ <sup>2</sup>	13	..	13	2	..	..	..	..	..	..	..	..	..	..	..	6	34	..	13	2
32.207	d	1	14	3	5	..	..	10	6	..	..	..	..	..	..	..	..	39	30	13	11
32.208	d q × cn σ <sup>2</sup>	2	..	..	2	..	..	..	..	..	..	..	25 <sup>f</sup>	9	5	..	..	43	..	24	7
32.209	l in F <sub>1</sub> d q × cn σ <sup>2</sup>	18	..	..	27	5	..	..	..	..	..	..	..	..	..	..	..	50	..	..	32
32.210	cn f	22 <sup>g</sup>	..	27 <sup>g</sup>	..	..	..	..	..	..	..	..	..	..	..	..	..	49	..	27	..

TABLE 2 (Continued)

POPULATION	PARENTAL PHENOTYPE	n	d	f	sl	m	df	dm	fm	lm	cr	ccv		NEW TYPES	TOTAL	TOTAL			
												n	l			d	f	m	
32.211	f	24	..	23	..	..	..	..	..	..	..	..	..	..	3	50	..	23	
32.212	n in f line	47	..	2	..	..	..	..	..	..	..	..	..	..	1	50	..	2	
32.213	l	21	..	2	11	4	..	..	..	..	..	..	..	..	6	44	..	2	
32.214	l	15	..	10	13	1	1	..	..	..	..	..	..	..	3	43	..	10	
32.215	sl	33	..	2	12	3	..	..	..	..	..	..	..	..	..	50	..	2	
32.216	n in l line	50	..	..	..	..	..	..	..	..	..	..	..	..	..	50	..	..	
32.217	l	17	..	6	24	..	..	..	..	..	..	..	..	..	2	49	..	6	
32.219	d q X cr d	6	..	..	1	..	..	..	..	..	5	2	1	..	1	16	..	5	
32.220	n	34	..	..	..	15	..	1	..	..	..	..	..	..	..	50	..	..	
32.221	ml	1	..	1	5	..	..	..	..	1	..	..	..	..	..	8	..	1	
32.222	ccv	9	2	1	2	..	..	..	..	..	..	6	..	..	..	20	2	1	
32.223	ccv q X cr d	3	..	..	7	..	..	..	..	..	34	2	3	..	..	49	..	34	
32.224	ccv q X cr d	10	..	..	6	..	..	..	..	..	23	1	2	..	1	43	..	23	
32.225	n	50	..	..	..	..	..	..	..	..	..	..	..	..	..	50	..	..	
32.226	n in F <sub>1</sub> cn q X d d	44	..	..	..	6	..	..	..	..	..	..	..	..	..	50	..	..	
32.227	l in F <sub>1</sub> cn q X d d	26	..	10	11	..	..	..	..	..	..	..	..	..	2	49	..	10	
32.228	sl in F <sub>1</sub> cn q X d d	14	..	8	10	3	..	..	..	..	..	..	..	10*	4	49	..	8	
Total		847	119	315	278	39	48	12	18	24	26	14	90	34	12	35	58	1969	171
																		415	357

\* Classification into *fluted* and *long-flowers-mammoth* not completed because of delayed blossoming.

b Some of these were *deformed-fluted* and *deformed-long-flowers-mammoth*.

c These mammoth have been classed as *fluted*, but their delayed blossoming has prevented verification.

d See description.

e See description.

f One plant with coral flowers was not *fluted*.

g All with coral flower color.

Abbreviations used: ccv = carmine-coral variegation; cn = control; cr = coral; d = deformed; f = fluted; l = long-flowers; m = mammoth; n = normal; sl = super-long-flowers; ds = variants.

ously growing young plants of *N. tabacum* (Commercial Havana No. 38) as test plants gave negative results after holding for 30 days. We are indebted to Dr. W. TAKAHASHI of the Division of Plant Pathology, UNIVERSITY OF CALIFORNIA, for carrying out these tests. In view of the negative evidence which they gave and the character of the genetic evidence, it did not appear necessary to test the possibility of transmission following grafting. Apparently *deformed* is a condition comparable in its expression to STEIN's "farb- und formdefekt" types (STEIN 1926) and her "Phytocarcinome" (STEIN 1932) for it involves a mosaicism of light and dark areas in the leaves as well as other evidence of irregular tissue development (figure 1).

#### MATERIALS AND METHODS

The preceding chart (table 1) traces the ancestry of our present *deformed* plants to the original  $X_1$  plant (28.303P51) already described. Approximately 2000 plants (table 2) have been grown under the designations given in this chart, and more than 100 have been studied cytologically.

In addition to 28.303P51, a number of other plants exhibiting deformities of various types have appeared in  $X_1$  or in later generations. While we do not feel justified in considering each of these reappearances as equivalent to the original *deformed*, nevertheless all our types are undoubtedly reflections of somatic chromosome elimination.

Most of the cytological study of plants in the *deformed* group was made on meiotic divisions in PMC. EMC were also used, and root tips were examined to obtain evidence as to the chromosome behavior in somatic mitoses. For PMC, iron-aceto-carmines were found most satisfactory. Permanent brazilin and aceto-carmines slides were made, following WEBBER's (1929) method, and STEERE's (1931) modification of McCLINTOCK's method. Anthers and ovaries showing meiotic stages were also fixed in Navashin's fluid, the anthers being first dipped in Carnoy's solution. Root tips were fixed in Navashin. Paraffin sections were stained with iron-alum haematoxylin or crystal violet. The drawings (figures 10-23) were, as indicated in the legends, made from slides prepared according to the various techniques just mentioned. The differences in magnification noted are referable to the effects of the various techniques on chromosome size.

#### GENETICS OF DEFORMED

A striking genetic characteristic of all *deformed* plants so far tested has been their consistent production of progenies containing plants belonging to five or more distinct morphological types (table 3 and figure 4). Plants of each of these types derived from *deformed* have become the sources of



races which differ significantly in their genetic behavior. All of these races have been characterized by continued segregation of types referable to quantitative variation in the *fluted*, or F chromosome. The X<sub>3</sub> progeny from *deformed* shown in figure 5 contains such a series of types.

*Deformed* plants transmit the *deformed* condition to approximately half their progeny upon selfing, as shown in table 3.

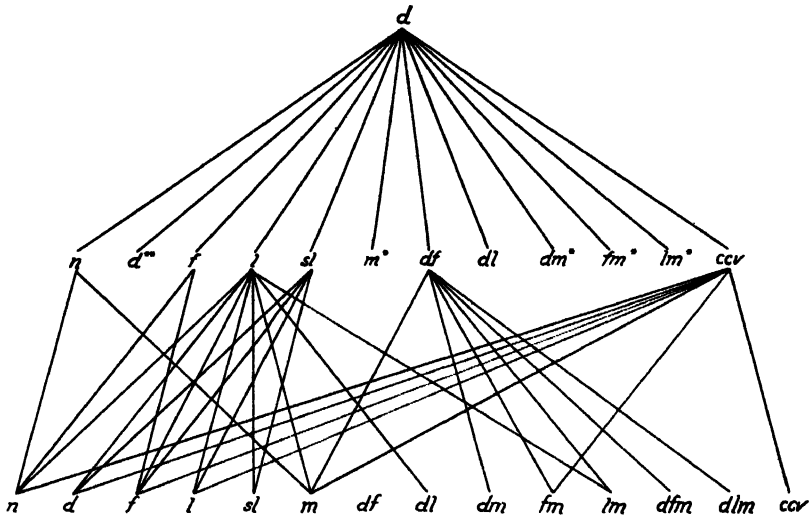


FIGURE 4.—Transmission of types derived from *deformed*. (\* Segregation of types in progenies of *mammoth* has not been studied. \*\* *Deformed* segregates as before.)

TABLE 3  
*Transmission of deformed.*

POPULATION	n	d	f	l	m	df	dl	dm	fm	lm	TOTAL	TOTAL			
												d	f	l	m
29.137	6	30	5	7	2	..	..	..	..	..	50	30	5	7	2
30.113	6	16	4	2	..	1	2	..	..	..	31	19	5	4	..
30.115	4	17	15	9	..	..	4	1	..	1	51	22	15	14	2
31.205	5	13	9	3	1	..	..	2	1	1	35	15	10	4	5
31.212	6	25	14	11	4	..	3	..	2	4	69	28	16	18	10
32.207	1	14	3	5	..	10	6	..	..	..	39	30	13	11	..
Total	28	115	50	37	7	11	15	3	3	6	275	144	64	58	19

Thus in the X<sub>2</sub> progeny of 50 plants grown from the original *deformed* plant there were 30 more or less *deformed* plants. A *deformed* plant of this X<sub>2</sub> population gave 31 plants in X<sub>3</sub>, 19 of which were *deformed*. One of these X<sub>3</sub> *deformed* plants gave an X<sub>4</sub> of 35 plants, 15 of which were *deformed*, and one of these in turn gave an X<sub>5</sub> of 39 plants, 30 of which showed deformity. When we combine with these four populations the two others from *de*-

*formed* plants, the total number of *deformed* is approximately one-half of all the plants grown (144 in 275).

This transmission of *deformed* suggests that of a quantitative chromosome variation in that no pure-breeding *deformed* occur; and since *deformed* does not segregate in Mendelian ratios in  $F_2$  of *deformed*  $\times$  *control*, it is clear that

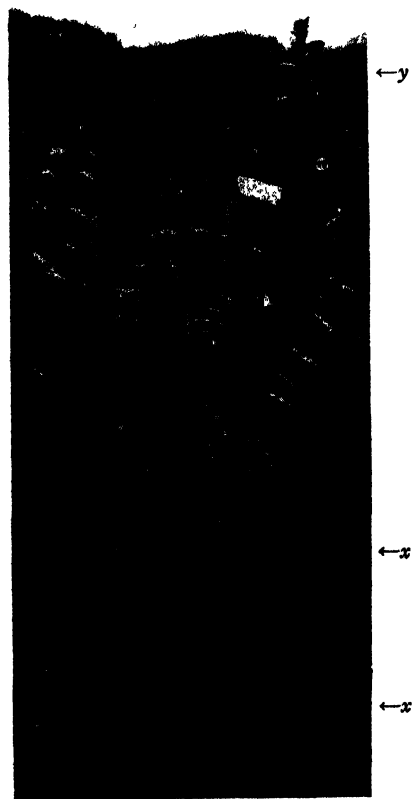


FIGURE 5.—Progeny of a *deformed* (30.115), showing two *deformed* ("x") in the foreground, and a *long-flowers-mammoth* ("y") in the background.

a monogenic mutation is not involved. Further, reference to tables 3 and 5 shows that *deformed* is not transmitted as a simple monosomic or trisomic condition, since *deformed*  $\times$  *control* gives no *deformed*. It is to be noted, however, that like these unbalanced types, *deformed* is transmitted to approximately 50 percent of its progeny. As will be shown in the discussion of the cytological evidence, this situation is produced by the operation of a mechanism involving chromosome attachment.

It is to be noted that the expression of the *deformed* condition does not become less pronounced in succeeding generations. It varies greatly in its expression in different individuals in the same population and in different parts of the same individual, but some of the plants in  $X_2$  were even more deformed than the original  $X_1$  plant (figure 6).

*Deformed* plants give *long-flowers*, *fluted*, and *mammoth* in their progenies and these may appear in combination with the *deformed* condition or with each other, to give the classes *deformed-fluted*, *deformed-long-flowers*, *de-*

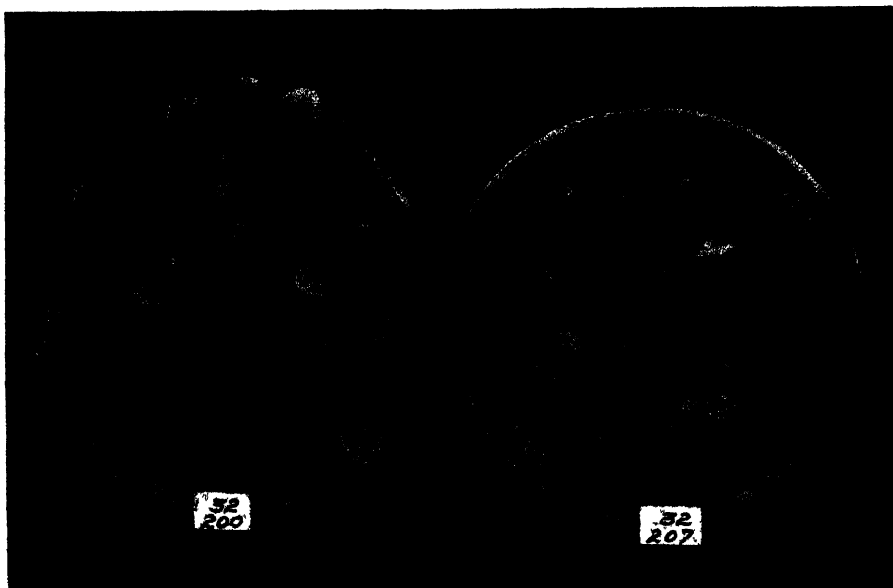


FIGURE 6.—Young seedlings in lines derived from *deformed*: 32.200, a population showing no *deformed*, and 32.207, a population in which a large number of the seedlings are *deformed*.

*formed-mammoth*, *fluted-mammoth*, *long-flowers-mammoth*, *deformed-fluted-mammoth*, and *deformed-long-flowers-mammoth* (figures 4 and 7). The classification of the progenies (table 3) of these *deformed* plants shows, in the first place, the great variety of forms obtained and, in the second place, the comparative similarity in the ratios of the types to one another in each population. In these populations the number of *long-flowers* is approximately equal to the number of *fluted*, and taking all the populations together, there are 58 *long-flowers* to 64 *fluted*. Of the total 275 plants, 144 or 52 percent are *deformed*; 58 or 21 percent are *long-flowers*; 64 or 23 percent are *fluted*; 19 or 7 percent are *mammoth*; and 28 or 10 percent are *normal*.

The classes of plants obtained in these progenies show clearly that the *fluted* (F) chromosome is present in different doses, for most of these types have been obtained under natural conditions when the F chromosome was

TABLE 4  
*Table of flower lengths in deformed populations (in mm).*

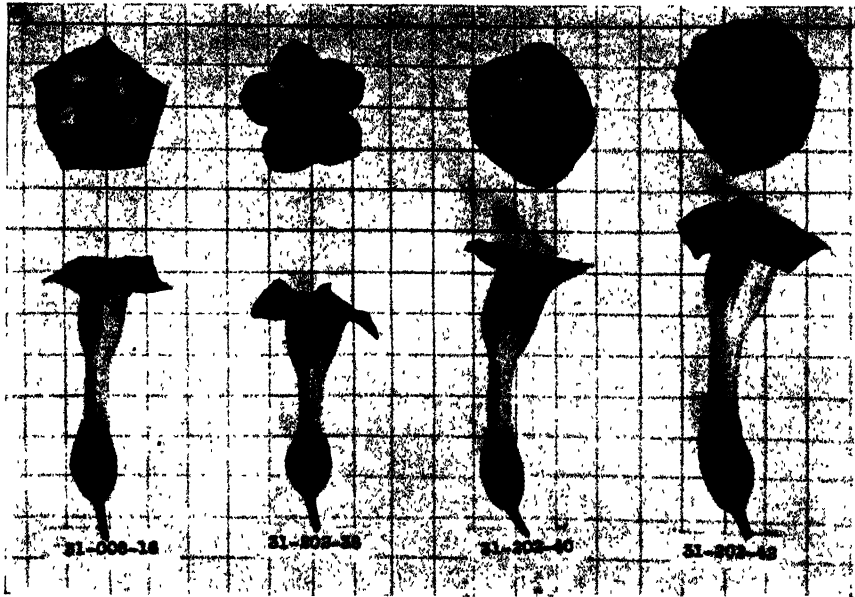
POPULATION	FLUTED							NORMAL										LONG-FLOWERS							SUPER-LONG FLOWERS				TOTAL
	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65			
29.137	..	..	1	1	..	1	..	..	1	..	2	3	4	..	7	8	4	..	..	..	..	2	1	..	1	1	37		
30.113	..	..	2	1	2	..	..	..	..	1	3	1	..	..	1	1	..	..	..	..	..	2	1	..	..	..	15		
30.115	..	4	4	3	1	4	..	1	..	1	2	3	2	..	1	1	1	1	1	1	2	6	..	..	..	3	42		
31.205	1	1	1	4	1	..	1	..	1	1	2	1	3	1	..	1	..	..	..	..	1	..	1	..	..	..	21		
31.212	3	2	5	5	1	1	1	..	..	1	2	2	3	3	2	..	3	3	..	1	3	1	2	..	..	2	46		
32.207	..	3	3	3	2	3	..	..	1	2	..	1	1	1	1	2	2	2	1	..	..	..	1	..	..	1	30		
Total	4	10	16	17	7	9	2	1	3	6	11	11	13	5	12	13	10	6	2	2	6	11	6	..	1	7	191		

present in abnormal number or constitution (CLAUSEN 1931). However, the ratios (table 3) in which these types occur here cannot be explained according to the genetic interpretations previously postulated in the case of un-X-rayed plants of the "*fluted assemblage*" (see CLAUSEN, l. c.). The unusual ratios obtained here point to the operation of a mechanism not previously encountered. Thus, the occurrence of both *fluted* and *long-flowers* plants in the progeny of *deformed*, a member of the *fluted assemblage*, is unexpected. *Fluted* and *long-flowers* appear in approximately equal numbers in such progenies, whereas, in un-X-rayed lines, *fluted* (monosomic F) gives *fluted* and *normal* (see CLAUSEN, l. c.) and *large-lax* (trisomic F) gives large flowered plants and *normal* in selfed progenies. Flower lengths in populations from *deformed* are given in table 4. The situation detailed there illustrates the F chromosome classes which occur.

Evidence that *deformed* produces gametes which vary quantitatively as regards the F chromosome from the nullo- to the triplo-F condition is furnished by the crosses of *deformed*  $\times$  *control*. It is significant that in these crosses no *deformed* have occurred. In comparison to the progenies obtained by selfing the same *deformed* plants, these populations show only a few classes of individuals: chiefly *normal*, *long-flowers*, and *fluted* (table 5). Two populations from *deformed*  $\varnothing \times$  *control*  $\sigma$  (31.206 and 32.205) and two populations of the reciprocal cross, but involving other *deformed* plants (31.213 and 31.215) have been grown. In each case more *fluted* were obtained when *deformed* was used as the female parent and more *long-flowers* when used as the male parent. This indicates that nullo-F female gametes are not as effectively eliminated by competition as are nullo-F male gametes. It is to be noted that where *deformed* was pollen parent, the progeny contained *fluted* and a large number of *long-flowers*, whereas these monosomic and trisomic conditions in un-X-rayed lines are transmitted only to a very small percentage through pollen (see CLAUSEN, l. c.). The population 31.213 is especially significant in this connection in that 21 of the total 25 plants were *long-flowers*. Here, *long-flowers* must be the result of transmission of excess chromosome material by the pollen of *deformed*.

TABLE 5  
*Progenies of deformed crossed with control.*

POPULATION	CROSS	n	d	f	l	dl	TOTAL
31.206	<i>d</i> $\varnothing \times$ <i>cn</i> $\sigma$	11	..	8	3	2	24
31.213	<i>cn</i> $\varnothing \times$ <i>d</i> $\sigma$	2	..	1	21	1	25
31.215	<i>cn</i> $\varnothing \times$ <i>d</i> $\sigma$	12	..	3	8	3	26
32.205	<i>d</i> $\varnothing \times$ <i>cn</i> $\sigma$	13	..	13	2	6	34
Total		38	0	25	34	12	109

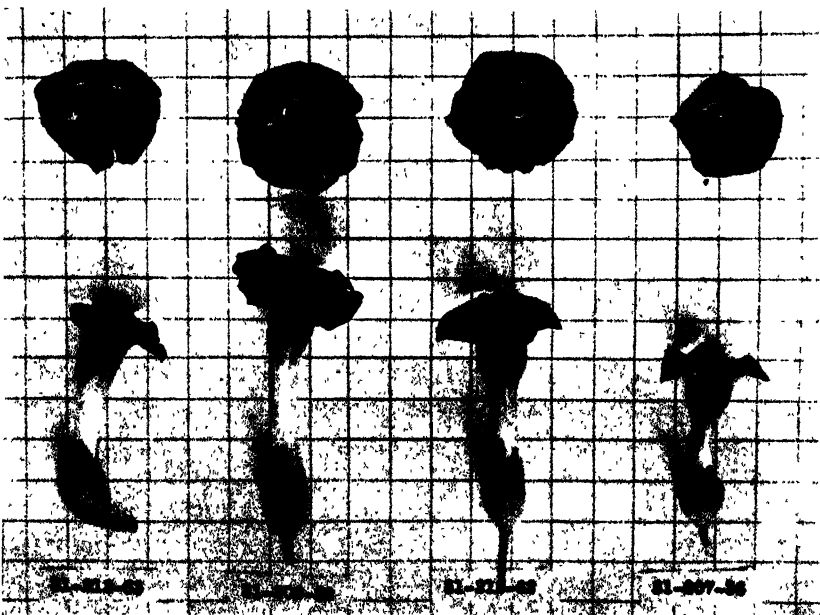


a

b

c

d



e

f

g

h

FIGURE 7.—Flowers of control and of lines derived from deformed: a, control; b, fluted; c, long-flowers; d, super-long-flowers; e, deformed; f, deformed-long-flowers; g, carmine-coral variegation; h, coral-fluted.

TABLE 6  
*Progenies of deformed and carmine-coral crossed with coral.*

POPULATION	CROSS	n	d	l	crf	ccv		sls	TOTAL
						n	l		
32.204	d ♀ × cr ♂	30	..	1	3	14	1	..	49
32.208	d ♀ × cr ♂	2	..	2	25*	9	5	..	43
32.219	d ♀ × cr ♂	6	..	1	5	2	1	1	16
32.223	ccv ♀ × cr ♂	3	..	7	34	2	3	..	49
32.224	ccv ♀ × cr ♂	10	..	6	23	1	2	1	43
Total		51	0	17	90	28	12	2	200

\* One plant with coral flowers was not *fluted*.

The fact that *coral* (CLAUSEN, l. c.) behaves genetically as a Mendelian recessive borne by the F chromosome has made it possible to analyse further the constitution of the functional gametes of *deformed* by means of crosses with homozygous *coral* plants. The results of three such crosses are shown in table 6. Where the F chromosomes of *deformed* have been eliminated from the gametes or zygotes, F<sub>1</sub> is *coral-fluted*, that is, it exhibits the morphological characters of *fluted* together with coral flower color. The number of *coral-fluted* plants thus becomes an index to the amount of F chromosome elimination. In the plants which are not *coral-fluted*, the occurrence of *carmine-coral* variegation indicates that somatic elimination of the F chromosome derived from the *deformed* parent is going on (figure 7, g). The type of variegation here is similar to that reported by CLAUSEN (1930) as due to elimination in mitosis of a fragment of the F chromosome. The degree of expression of *carmine-coral* variegation in different plants is highly variable, some showing only small coral flecks while others have whole branches bearing only coral flowers. As to flower size, the plants which show this variegation may be either *normal* or *long-flowers*.

The *coral* originally used in these crosses was not a *deformed* derivative, but more recently a *coral* race has been established in the *deformed* lineage. As shown in tables 1 and 2, 31.207 is a *fluted* line derived from an X<sub>2</sub> plant, and P36 of this culture was a small, *fluted* plant which bore only coral flowers (figure 7, h). From it a culture (32.210) has been obtained consisting of *normal-coral* and *coral-fluted* plants. In many other instances it has been clear that the alteration of the F chromosome productive of coral flower color has occurred, for plants in populations derived from *deformed* and into whose ancestry no *coral* has been introduced, have shown *carmine-coral* variegation. Thus 31.212P62 was a non-*deformed* plant of normal flower length (listed as *normal* in table 2), whose flowers showed considerable *carmine-coral* variegation (figure 7, g). It was selfed and crossed

twice with *coral*, different branches being used in each case. As is evident from table 6, the progenies resulting from these crosses with *coral* (32.223 and 32.224) resemble closely those resulting from crosses of *deformed* with *coral*. The selfed progeny also suggests that of *deformed*, for two typical *deformed* plants appeared in the progeny of this non-*deformed*. There were also several plants in this population which again showed *carmine-coral* variegation but no deformity, so that a somatic loss leading to a condition other than that responsible for *deformed* must have occurred.

From these observations concerning the genetic behavior of *deformed* when selfed and crossed with *control* and with *coral*, it is evident that:

(1) The *deformed* condition is transmitted from one generation to the next through selfing but not through crosses with the control.

(2) The mosaic and variable expression of tissue abnormality characteristic of *deformed* resembles the color variegation produced on crossing *deformed* with *coral* and indicates that diversity in the chromosome constitution of somatic cell lines is responsible for both of these expressions.

(3) Progenies of *deformed* selfed and crossed with *coral* consist of many distinct types, while in progenies from crosses with *control* there are relatively few distinct types.

There are apparently some significant differences in the genetic behavior of *fluted* and *long-flowers* derived from *deformed*. Thus *fluted* selfed gives the two classes expected, *normal* and *fluted*, while the progenies of *long-flowers* usually consist of three to seven classes and contain *fluted* as well as *long-flowers*. *Fluted* has never given *deformed*, while *long-flowers* has given *deformed*. These facts indicate that the segregation of *fluted* is accompanied by the loss of ability to produce the *deformed* condition and to produce progenies consisting of many distinct types, while *long-flowers* may retain both of these capacities.

There is a considerable difference in the number of *fluted* from *fluted* selfed (table 7), but the average of all the populations grown is so close to that found in untreated lines that this difference probably has no sig-

TABLE 7  
*Transmission of fluted from deformed.*

POPULATION	n	f	sts	TOTAL	PERCENT f
30.114	11	37	1	49	76
31.200	26	22	2	50	44
31.207	30	18	..	48	38
31.210	10	43	2	55	78
32.210	22	27	..	49	55
32.211	24	23	3	50	46
Total	123	170	8	301	56 (average)



nificance, especially since the percentage of *fluted* often varies widely in different untreated cultures. (The percentages here varied from 38 to 78, but the average of 6 populations was 56 percent, which is close to the percentage reported by CLAUSEN and GOODSPEED [1926] for the transmission of the *fluted* character in untreated cultures.) One exception to the usual mode of transmission of *fluted* occurred in the progeny (31.201) obtained from *deformed-fluted* (table 2). Here, instead of approximately one-half *fluted* and one-half *normal*, the whole progeny consisted of *mammoth*. Due to failure of these plants to blossom during the normal flowering period, their classification as regards flower-size into *long-flowers*, *fluted*, and *normal* was not completed. It was evident, however, that these three types did occur. Of 50 plants, 19, or a little less than half, were deformed, and some of these were also *long-flowers* or *fluted*. Here, the types appearing in connection with the *mammoth* character are the same as those in non-*mammoth* populations from *deformed*. Apparently the modification of the F chromosome necessary to produce the *mammoth* condition must have occurred in the *deformed-fluted* parent at some time before sex-cell formation, but after the character of the plant itself had already been determined by the non-*mammoth* producing F chromosome.

The same change in the F chromosome which led to the production of all *mammoth* in 31.201 must also occur in *normal* (not *fluted*) *deformed* plants since *mammoth* occurs in many progenies from *deformed*. Undoubtedly plants heterozygous for *mammoth* occur in these progenies but due to the complexity of types dealt with, they have not been classified with certainty, so that *mammoth* has only been dealt with when it has segregated in a homozygous state. Two exceptional *mammoth* types were included among the ten *mammoth* in 31.212. These plants had the characteristic growth habit of *mammoth* with the leaf type of *long-flowers-mammoth*, and were only distinct from other *mammoth* when they bloomed late in the season but several months before *normal-mammoth*. Both of these plants were long-flowered and their abnormal behavior in respect to the flowering season is possibly related thereto. If *long-flowers* is due to the presence of supernumerary F chromosomes and *mammoth* to alteration of this chromosome, these plants may have possessed two F's carrying the *mammoth* modification and one which did not, the result being a condition approaching *mammoth* but altered in the direction of *normal* as regards time of blooming. The progeny of one of these plants (32.221) bears on this assumption, for of 8 plants grown, 1 was *mammoth* and 5 were *long-flowers*.

Segregation for *mammoth* in two  $X_1$  cultures in three to one ratios, indicates that the change in the F chromosome responsible for the *mammoth* condition had been produced in  $X_1$  and transmitted to the parent plants which were undoubtedly heterozygous for *mammoth*. Thus 32.220 came

from a *normal* in a population from *deformed*, and consisted of 50 plants, 16 of which were *mammoth*. In the case of 32.200, which will be discussed later, 12 out of 50 plants were *mammoth*. The  $X_2$  parent of this  $X_3$  population was *fluted*, so that the change in the F chromosome responsible for the production of *mammoth* did not occur in the  $X_2$  *deformed* plant, but probably must have occurred following its presence in the grandparental zygote, since the parent plant was of *normal* habit and flower size. Six *mammoth* occurred among the 50 of 32.226, which was the  $F_2$  of a *normal*  $F_1$  from *control*  $\times$  *deformed*. Thus *mammoth* is constantly being produced and constantly segregating from *deformed* and in genetic behavior resembles a recessive gene mutation.

As already mentioned, the genetics of the long-flowered types is not as simple as that of *fluted* derived from *deformed*. The mode of origin of *long-flowers* as well as its subsequent behavior indicates that it is a reflection of the presence of at least a part of the F chromosome in excess of its normal diplo-condition. CLAUSEN (1931) has shown that triplo-F is a large-flowered type, morphologically close to the trisomic "enlarged," and he calls this form "*large-lax*". Our *long-flowers* is close to *large-lax* in its morphological features but genetic tests have not been made which identify the former type with the F chromosome. It is possible that some other chromosome, such as the *enlarged* chromosome, may be concerned in the production of at least some of the long-flowered types derived from *de-*

TABLE 8  
*Transmission of long-flowers types.*

POPULATION	n	d	f	l	sl	m	dl	ml	sta	TOTAL	TOTAL			
											d	f	l	PERCENT l
31.202	3	2	11	14	7	2	3	3	..	45	5	11	27	60
31.208	16	..	2	26	4	..	1	1	..	50	..	2	32	64
31.209	17	..	12	20	..	..	..	..	..	49	..	12	20	41
32.203	23	..	..	16	10	..	..	..	..	49	..	..	26	53
32.213	21	..	2	11	4	..	..	..	6	44	..	2	15	34
32.214	15	..	10	13	1	1	..	..	3	43	..	10	14	33
32.215	33	..	2	12	3	..	..	..	..	50	..	2	15	30
32.217	17	..	6	24	..	..	..	..	2	49	..	6	24	49
32.209*	18	..	..	27	5	..	..	..	..	50	..	..	32	64
32.227*	26	..	10	11	..	..	..	..	2	49	..	10	11	22
32.228*	24**	..	8	10	3	..	..	..	4	49	..	8	13	27
Total	213	2	63	184	37	3	4	4	17	527	5	63	229	43 (aver- age)

\* Progeny of  $F_1 \delta \varphi \times cn \sigma^7$ .

\*\* 10 were somewhat longer than normal, but did not belong to the *long-flowers* class.

formed. We believe, however, that the genetic analysis is adequate and simpler, if these types are assumed to be produced by the presence of excess F chromosome material.

As is shown in table 8, 9 of the 11 progenies of *long-flowers* so far grown have contained *fluted* as well as *long-flowers*, which is unexpected since monosomics are rare in progenies of trisomics. The progenies obtained from *long-flowers* suggest those obtained from *deformed*, for occasionally *mammoth* and *deformed* as well as *long-flowers* and *fluted* occur. The percentage of *long-flowers* from *long-flowers* selfed varies from 22 to 64, the average for all populations being 43 percent (table 8). The plants in this *long-flowers* class fall into at least two distinct flower-length groups: the first 58-62 mm long; the second, *super-long-flowers*, 63-70 mm long. Three populations contained no *super-long-flowers* but did contain a considerable number of *fluted*. On the other hand, two populations contained no *fluted* but higher proportions of *super-long-flowers* than did the other populations. (31.202 contained a higher proportion of *super-long-flowers* together with a considerable number of *fluted*. The genetic and cytological evidence indicates that while the parent of this population was phenotypically *long-flowers*, the genotype transmitted through the gametes was that of a *deformed*.)

*Super-long-flowers* resembles *super-enlarged* in flower length but differs from it in other morphological characters. *Super-enlarged* is known to be a tetrasomic form and has been reported by CLAUSEN and GOODSPEED (1924) to give 20 percent of *super-enlarged* and 77 percent of *enlarged* in its progeny. It is therefore significant that *super-long-flowers*, 31.208P50, gave no more *super-long-flowers* in its progeny (31.215) than did other *long-flowers* not of the super-long class. It also gave two *fluted*. A *super-long-flowers* in F<sub>1</sub> of *deformed* × *control* also showed this same type of behavior, giving 3 *super-long-flowers*, 10 *long-flowers*, and 8 *fluted* in a population of 49 plants. These facts indicate that while *super-long-flowers* may in some cases represent a tetrasomic type, in other cases it is not due to the presence of four identical and independent chromosomes.

In certain populations, *long-flowers* shows considerable variation as to habit and flower-width, but this difference is not associated with differences in genetic behavior. Thus three categories of *long-flowers* in 31.208, one being the *super-long-flowers* already mentioned, gave approximately the same percentage of *long-flowers* in their progenies (32.213, 32.214, 32.215, table 8). By contrast, 31.209 contained *long-flowers* of only one type, and in the progeny of one of these (31.209P43) the *long-flowers* plants again all appeared to be equivalent (32.217).

Another type which sometimes appears in *long-flowers* populations and is less distinct than the super-long type, has flowers somewhat longer than

*normal* but not as long as *long-flowers*. Probably the plants in this group do not represent a uniform type for they show considerable variation in habit and flower shape. In two cases, however (32.201 and 32.228), the plants of this type were distinct from the rest of the population and have been classed together as a new type in table 2. In other populations where this type was not distinct, the plants have been recorded as variants.

In its transmission, as well as in its morphological expression, *long-flowers* resembles a trisomic type such as *enlarged* or *large-lax*. It differs from the trisomic type, however, in the considerable percentage of monosomics produced and in the genetic behavior of some plants of its extreme type, *super-long-flowers*.

TABLE 9  
*Progenies of normal from deformed.*

POPULATION	n	f	m	sta	TOTAL
31.204	46	..	..	2	48
32.202	47	..	..	3	50
32.212	47	2	..	1	50
32.216	50	..	..	..	50
32.220	34	..	15	1	50
Total	224	2	15	7	248

Five populations have been grown from plants classed as *normal* which occurred in *deformed* progenies (table 9). Two of these populations were derived from *normal* plants in immediate progenies of *deformed* (31.204 and 32.220) while a third (32.202) carried one of these *normal* lines (31.204) to the  $X_6$  generation. Of the other two populations, one (32.212) came from a *normal* in a line which had been segregating for *fluted* for three generations, while the last (32.216) came from a *normal* in a line segregating for *long-flowers*. These five populations in contrast to all others from *deformed*, were uniform in habit, growth rate, and morphological features. Their fertility was so much greater than *deformed*, *fluted*, and *long-flowers* that they had completed their blooming period much before other types. Aside from the *mammoth* in 32.220, which have already been discussed, few variants occurred. The occurrence of two *fluted* in 32.212 may be ascribed to the fact that this culture was derived from *fluted*. It is therefore clear that pure breeding races phenotypically equivalent to *control* may be derived from *deformed*. It is probable, however, that even if the same numbers and kinds of genes are present in these plants as are present in the *control*, their structural organization may be quite different, due to transformations which have occurred in their *deformed* ancestry. Thus, these various *normals* from *deformed* might give different genetic results in crosses with *control*, a point which is being determined.

That plants which are close to *control* phenotypically may be genetically quite different was shown in the case of 31.200P8. It was of normal habit and possessed flowers of normal size (52×33 mm) which, however, suggested *fluted* in shape and showed a small amount of *carmine-coral* variegation. An extended series of *fluted* "plus" and "minus" types occurred in 32.200, the selfed progeny of this plant. Aside from 12 plants which did not differ significantly from *normal*, 3 *fluted* and 12 *mammoth* (presumably *fluted-mammoth*) are the only plants in this population which fall readily into known F chromosome types. The remainder of the population consisted of plants differing from *fluted* in greater or lesser degree. Thus 15 plants possessed erect close-ranked leaves typical of *fluted* but had flowers which were close to *normal* in length, rather than shorter as in *fluted*, and differed from *fluted* in flower shape. Six other plants resembled *fluted* in flower shape or size, or in both, but differed from *fluted* in some other morphological feature. One plant was close to *fluted* in habit and in flower shape and size but differed in leaf shape and showed *carmine-coral* variegation together with a small amount of white flecking of the corolla characteristic of *deformed*. One plant was long-flowered with a long petiolate leaf-base. Such a collection of *fluted* variants is a clear demonstration that the characteristic *fluted* morphological complex is being subdivided through production of viable by-products of alteration, the reflection of which has not before been reported.

#### CYTOLOGICAL EVIDENCE

The interpretation of the genetic results just discussed is based upon the following propositions derived from cytological evidence concerning the chromosomal constitution of *deformed* and its products. (1) The X-ray dosage, applied at prophase to the egg from the fertilization of which with an untreated pollen grain the original *deformed* plant arose, produced an "attachment" between two homologous chromosomes, presumably the two F's. "Attachment" in this sense involves a permanent physical union between chromosomes, with the result that disjunction of the pair involved does not occur at IA (figure 8). (IM, IA, IT = metaphase, anaphase, and telophase of first meiotic division; IIM, IIA, IIT = corresponding stages of the second meiotic division.) In origin "attachment" may be thought of as differing from translocation in the important point that whole chromosomes are "attached," producing a single unit with two spindle-fiber insertions. The original interpretation (GOODSPEED and AVERY, 1930, p. 313) given to this product of attachment was that "opposite ends of a single whole chromosome have become united each to one member of a pair with which the single chromosome is not homologous." While it is possible that chromosome material from some other chromosome than

the F was involved in the transformation leading to the attachment of the F chromosomes, the genetic data at present indicate that only the F chromosomes are concerned. The present interpretation of the cytologically observed attachment and its products involves such a union of F chromosomes as is shown in figure 8, a. The structure and behavior of the attached

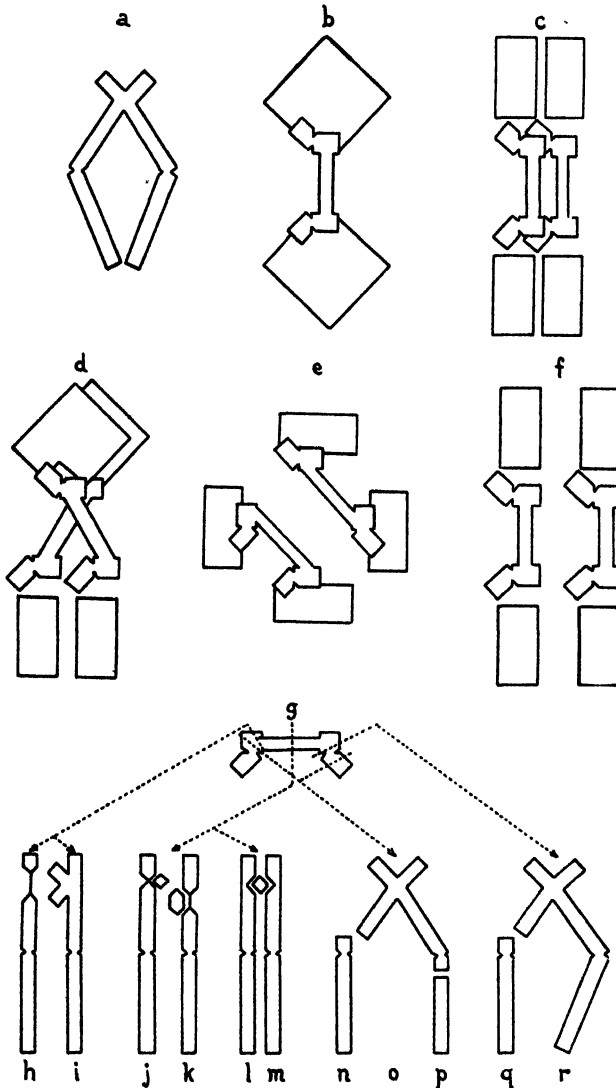


FIGURE 8.—Diagrammatic representation of behavior of attached F chromosomes in meiosis: a, diplotene; b, IIM, following the distribution of parts of the two F chromosomes to opposite poles; c, IIA, showing division of attached F's; d, e, f, IIT, showing three different orientations of the granddaughter nuclei, in each case chromatin bridges connecting two nuclei whose chromosomes were not derived from the same IIM nucleus; g, condition of the attached chromosomes at IIT, the dotted lines showing points of breakage of the chromatin bridge, to give rise to the products h to r.

units corresponds to this interpretation. If the force repelling the two homologous chromosomes at IA is sufficient to produce the chromatin bridge by attenuating the chromatin of the two chromosomes involved, the difference in degree and place of attenuation of the bridge leads to different apparent points of attachment as seen at IT to IIT, and this fact was responsible for the earlier interpretation. While part or all of this earlier interpretation may be valid, we have preferred to use the simpler

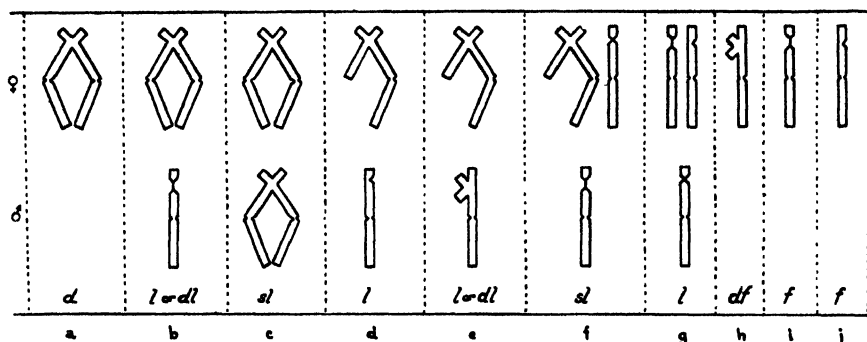


FIGURE 9.—Diagrammatic representation of the F chromosome constitution of some of the gametes of *deformed* and of their union to give the following types which occur in progenies of *deformed*: a, *deformed*; b, *long-flowers* or *deformed-long-flowers*; c, *super-long-flowers*; d, *long-flowers*; e, *long-flowers* or *deformed-long-flowers*; f, *super-long-flowers*; g, *long-flowers*; h, *deformed-fluted*; i and j, *fluted*.

one here described. (2) The chromosome behavior of such a unit is thought of in terms of duality in spindle-fiber insertion and, particularly, so far as meiosis is concerned, of breakages which occur at IIA. During mitosis the relation of this unit to the spindle will, at times, result in its lagging and

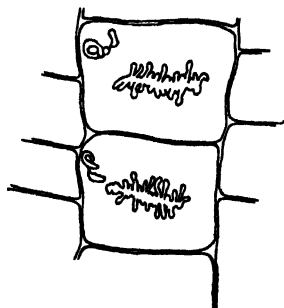


FIGURE 10.—Two adjacent cells from a longitudinal section of a root of *deformed*. In each cell a complex unit is distinct from the metaphase plate. For comment, see text (p. 509). (Paraffin;  $\times 1550$ .)

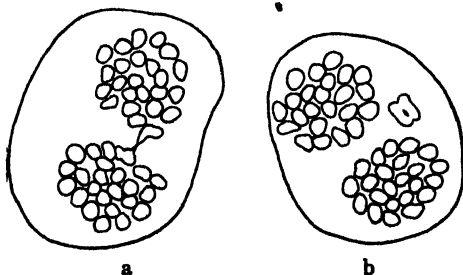


FIGURE 11.—IIM in an  $X_2$  *deformed* plant (29.137P14): a, 24 chromosomes in each plate, the F chromosomes having been distributed to opposite poles at IA, but remaining attached by a chromatin bridge; b, 23 chromosomes in each plate, the attached F chromosomes lagging between the daughter nuclei. (Paraffin;  $\times 1550$ .)

elimination (figure 10). (3) Products of IIA behavior are normal F's, deficient F's, and F's with various amounts and kinds of additions (figure 8, h-r). (4) Elimination in mitosis of this unit produced by attachment gives rise to abnormalities in tissue production and alignment. Certain of these points are illustrated by the diagrams in figure 8.

The mosaic-like expression of the *deformed* condition is a reflection of somatic elimination of F chromosomes as a result of their attachment. The attached chromosomes may be left in the plasma at telophase of mitoses in *deformed* plants, due to the mechanical difficulties sometimes arising in the separation and distribution of the products of division of the complex unit they constitute. Thus, figure 10 shows two adjacent cells, both in metaphase, from a longitudinal section of a root of a *deformed* plant. Here, in each cell, is a complex double unit, distinct from the metaphase plate, and beginning to degenerate. The fact that these units in adjacent cells appear to be structurally identical, indicates that they are products of delayed division of a complex unit which lagged in the preceding anaphase. The ultimate result of such elimination is the tissue degeneration which can be seen to occur in limited regions of most growing points of *deformed* plants. Nullo-F plants are not known to occur, due presumably to their zygotic lethality, and, similarly, tissue degeneration characteristic of *deformed* probably is a product of the nullo-F condition. The fact that the *deformed* condition has been preserved through five generations on selfing of *deformed* plants (figure 6) indicates that the attached unit, although it is being periodically eliminated in mitosis, is present in most if not all cell lines which are capable of terminating their mitotic cycle in sex-cell formation. Apparently the degrees of expression of the *deformed* condition depend upon the amount of elimination in early growth stages. Thus, the large amount of inviable seed and of death in earlier or later seedling stages which occurs, is a reflection of early and considerable elimination, with the result that not a sufficient amount of normal tissue is available to permit maturity to be attained.

Cytologically, the attached F's present in *deformed* plants are most conspicuous at meiosis because of their abnormal anaphase behavior (figure 8). At IM, the product of attachment may be indistinguishable from other bivalent chromosomes, but at IA the situation involved becomes evident because of the lack of disjunction of the two attached members (figure 11, a). Now, within the normal *tabacum* set, the F chromosome is long with median spindle-fiber insertion (CLAUSEN 1930). The attachment under discussion involves a union of the two F's at a point between the spindle-fiber insertion and one end, as shown in figure 8, a. If attachment was induced at an early meiotic stage, as seems probable, it might represent a permanent union at a point of chiasma formation.



The failure of disjunction of the two attached chromosomes at IA is evidenced by three different types of end product at IT. Most frequently separation of portions of the attached F's takes place, and, along with products of normal distribution, these portions are present in the daughter nuclei. Because of the attachment of the two F's, however, a chromatin

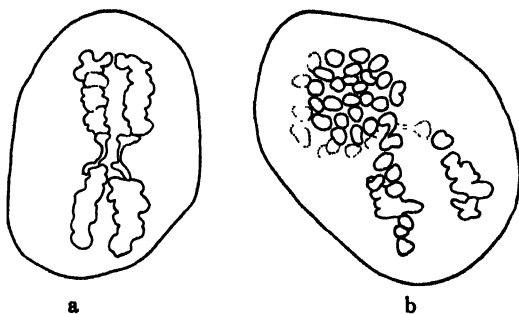


FIGURE 12.—IIA in an  $X_2$  *deformed* plant (29.137P14). The attached chromosomes have divided and form a chromatin bridge between nuclei whose chromosomes were not derived from the same IIM nucleus. Two different orientations of the four granddaughter nuclei are shown in a and b. (Paraffin;  $\times 1550$ .)

bridge is formed at IT (figure 11, a). In other cases the attached F's fail to reach either pole and lag between the daughter nuclei, where they may be seen as a complex double unit (figure 11, b). At other times, the entire product of attachment goes to one pole, or rarely, the strain caused by the distribution of portions of the two chromosomes to opposite poles may result in the breaking of the attachment at IT. Either of these latter two

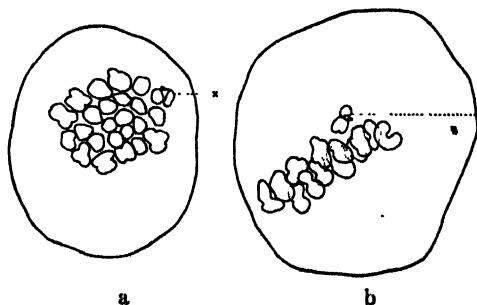


FIGURE 13.—IM in *fused*, derived from *deformed* (31.207P46): a, IM, polar view with 23<sub>II</sub> plus the F chromosome ("x"), which is of abnormal shape; b, IM, side view (all chromosomes not shown), F chromosome above the plate ("x"). (Aceto-carmine;  $\times 1140$ .)

types of behavior give IT and IIM plates which show neither a connection nor a lagging unit. The relative frequencies of these alternative behaviors were determined in the case of an  $X_2$  *deformed* to be 94:56:56, or approximately 2:1:1.

The critical period for the survival of the attached F's in their quanti-

tatively unaltered condition is, however, from IIM to IIT, when the four granddaughter nuclei are becoming most widely separated in the cell. Thus, if the attached units have been distributed to the same pole at IA, they may enter the gamete unaltered. If, on the other hand, they have been distributed to opposite poles at IA, at IIM the connecting chromatin

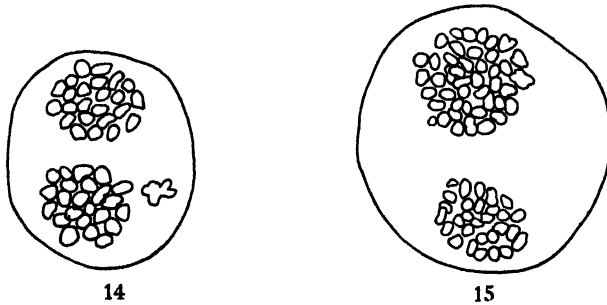


FIGURE 14.—IIM in *long-flowers* derive from *deformed* (31.209P33) showing 23 and 24 chromosomes at IIM and the F chromosome with attached segment lying in the plasma. (Aceto-carmine;  $\times 1140$ .)

FIGURE 15.—IIM in triploid from *deformed* (31.212P19), showing 28 plus a fragment in one plate, and 43 plus a fragment in the other. (Aceto-carmine;  $\times 1140$ .)

bridge will, itself, divide along with the chromosomes. As the daughter halves separate at IIA, two chromatin bridges will be formed, each connecting two nuclei whose chromosomes were not derived from the same IIM nucleus (figure 8, c; 12). The stresses under which the bridges are placed as the nuclei separate, produce breakage. The various regions of fracture are apparently determined by the relation of the point of attachment to the IIA forces operating in the cell.

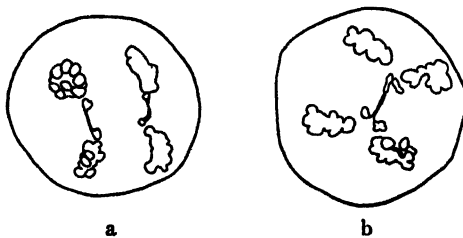


FIGURE 16.—IIT in *long-flowers* (30.115P12), showing chromatin bridges connecting nuclei as in *deformed* (see fig. 12). (Brazilin;  $\times 1140$ .)

At IIT, the products of breakage are strikingly apparent (see GOOD-SPEED and AVERY 1930, Plate 19, figures 10–14). Thus, if the break occurs near one spindle-fiber insertion, two units result, one of which is equivalent to half of an F chromosome, and the other to an F chromosome with an additional half attached (figure 8, q, r). If the break occurs near the point of initial attachment, one of the resulting F chromosomes may have a portion deleted, while the other will have this segment added (figure 8,

h, i). It is also possible that a double break may result in the production of a unit consisting of the same two halves of the F chromosome attached (figure 8, n, o, p). As to the other two halves, they are lost or preserved as larger fragments, depending upon whether or not they possess spindle-fiber insertion regions. In addition, very small fragments are produced following the breaking of the bridge (figure 8, j-m). These various products of the action of the IIA and IIT forces upon the chromatin bridges

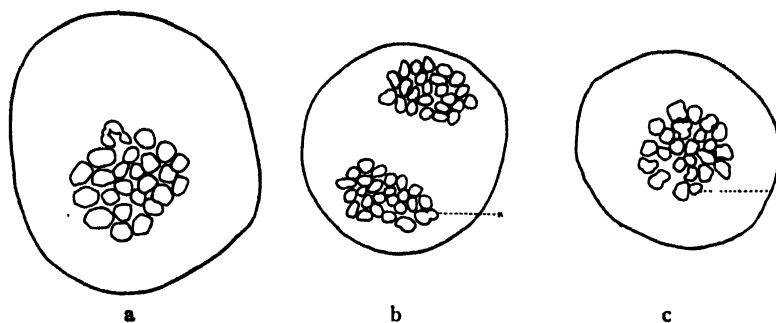


FIGURE 17.—PMC from two shoots of a chimera (32.217P4), showing different chromosome constitutions: a, IM in *long-flowers* shoot,  $23_{II}+1_{III}$ ; b, IIM in *long-flowers* shoot, 24-25; the chromosome marked "x" has an attached segment; c, IM in short-flowered shoot,  $23_{II}+1_I$ , the univalent marked "x." (Aceto-carmin;  $\times 1275$ .)

correspond closely to the kinds of F chromosomes found in progenies of *deformed* plants. Probably none of these products is structurally equivalent to an F in *control*, and yet genically it may be more nearly equivalent to such a unit. Thus, the genetic evidence shows that the F in *fluted* from *deformed* is genically close to normal F, and yet, as seen at IM in these plants, this unit, conspicuous in the monosomic condition, is not struc-

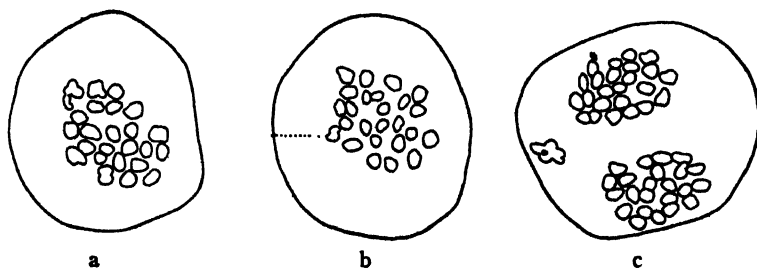


FIGURE 18.—Meiosis in *deformed-long-flowers* (30.115P27): a, IM,  $23_{II}+1_{III}$ ; b, IM,  $24_{II}+1_I$ , the univalent marked "x"; c, IIM, 23 chromosomes in one plate, 24 in the other, and an F with attached segment in the plasma. (Brazilin;  $\times 1140$ .)

turally equivalent to normal F. Thus, as shown in figure 13, it occurs as two discrete segments physically united at one point. The chromosomes present in certain *long-flowers* derivatives are even more strikingly distinct from normal F, for here there is often a large segment attached to an other-

wise normal chromosome (figure 14). The derivation of such chromosomes from the unit present in *deformed* is shown in figure 8, g-r. In *deformed* plants it has been observed that the connection of IIM plates by means of the attached F's may lead to the formation of "restitution nuclei," and thus of somatic gametes. Two plants from *deformed* have proved to be triploids, 31.212P19 (figure 15) and 32.207P1 (GOODSPEED 1930).

The fact that *deformed*  $\times$  *control* gives no *deformed*, but gives *long-flowers* and *fluted*, is further evidence of the chromosomal mechanism operating in the production of gametes in *deformed* plants. The non-appearance of *deformed* is due to the contribution by the *control* of a normal F chromosome. If the *deformed* gamete contributes a single F derived from such a breaking of the bridge, as is shown in figure 8, l, m, a *normal* plant is produced. The occurrence of *fluted* in progenies of *deformed*  $\times$  *control* is the result of the lack of the entire attached unit in the egg. On the other hand, the long-flowered plants in such progenies are, as noted above, triplo-F. Obviously there is a possibility that such a plant contains three unattached F's as a result of the inclusion of both products of the break at IIM in the production of the egg (figure 9, g). If the *deformed* egg contains attached F's, the addition of a normal F from *control* determines lack of deformity and gives *long-flowers* (figure 9, d). Thus, in this latter case, although the attached F's are undoubtedly present and being eliminated in mitosis as in *deformed*, the unattached F prevents the degeneration of the cells involved and thus the external evidence of tissue deformation (figures 16, a and b).

That chromosome elimination takes place in somatic tissues of *long-flowers*, was made evident in the progenies from *deformed*  $\times$  *coral*, where the structurally unaltered F contained the recessive flower color factor, while the dominant carmine factor was introduced with the F chromosome complex from *deformed*. In these progenies, the elimination of the F chromosome from the gametes of *deformed* could be measured by the number of *coral-fluted* plants, and the elimination of F chromosomes derived from *deformed* in somatic mitoses could be followed by the occurrence of *carmine-coral* variegation (figure 7, g). This kind of variegation occurred in *long-flowers*, as well as in plants with flowers of normal length, and is an indication that the attached chromosomes present, responsible for *long-flowers*, are occasionally eliminated in somatic divisions.

Further evidence that chromosome elimination takes place in *long-flowers* without producing deformity, is found in the fact that three different plants of this phenotype have given rise to shoots from the base of the central stalk on which all the flowers had lost the *long-flowers* character, due to the loss of one or more F's from the cells of their central axes giving rise to these shoots. In the case of 32.217P4 the loss could be ob-

served cytologically by comparison of the PMC produced by the central axis and the side shoot. Thus,  $23_{II}+1_{III}$  at IM (figure 17, a) and 24–25 at IIM (figure 17, b) were the characteristic configurations in PMC from the *long-flowers* axis, while the PMC from the short-flowered shoot showed only  $23_{II}+1_I$  (figure 17, c).

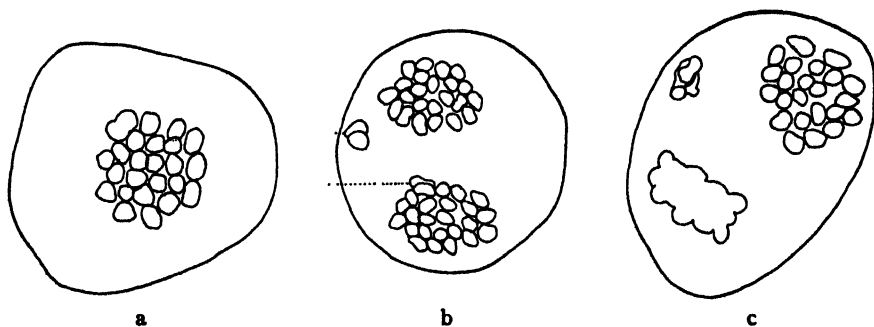


FIGURE 19.—Meiosis in *super-long-flowers* (32.209P39): a, IM,  $23_{II}+1_{IV}$ ; b, IIM, 25 chromosomes in one plate, two of which were not disjoined at IA ("x"), 23 in the other plate, and two products of non-disjunction in the plasma ("y"); c, IIM, 23 plus a quadrivalent in the plasma. (Aceto-carmin;  $\times 1230$ .)

The genetic evidence given in table 3 shows that non-*deformed* lines of *fluted*, *long-flowers* and *mammoth* have been derived from the original *deformed* plant. The origin of such lines is as follows. Where the attached unit is entirely absent in a gamete, due to elimination in meiosis, the gamete being therefore nullo-F, its union with a haplo-F gamete, produced through

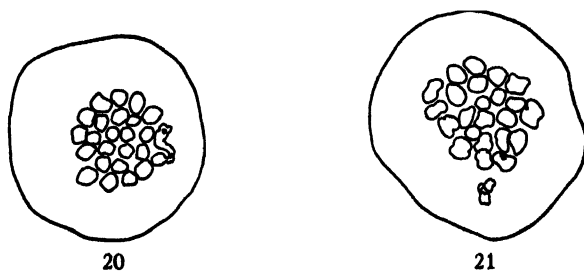


FIGURE 20.—IM in *super-long-flowers* (31.208P50), showing  $23_{II}$  plus a quadrivalent involving a "chain" of chromosomes. (Aceto-carmin;  $\times 1140$ .)

FIGURE 21.—IM in *fluted-mammoth* (31.212P32);  $23_{II}+1_I$ , the chromosome in the plasma showing a type of structural abnormality of the F chromosome. (Aceto-carmin;  $\times 1140$ .)

breaking of the attachment, gives *fluted* (figure 9, i and j). On the other hand, *long-flowers* represents essentially the triplo-F condition. It may be a product of the union of a gamete containing the attached unit and a haplo-F gamete derived as in the case of *fluted* mentioned above (figure 9, b). At IM such a plant will show either 24 or 25 units. In the former case one unit is structurally equivalent to a trivalent, two members of which

are attached (figure 18, a); in the latter there is a univalent not associated with the attached unit (figure 18, b). When the attachment is broken to give two normal F's, their union with a normal gamete will also give *long-flowers* (figure 9, g). Again, when the breaking of the attachment is such that a segment of an F is still attached to the other F, so that the segment lacks a spindle-fiber insertion, a gamete results which, united with a gamete containing a normal F, gives *long-flowers* (figure 9, d). A plant so derived gives evidence of its chromosome constitution at IIM, where the two plates contain respectively 23 and 24 chromosomes with a unit in the plasma, the latter showing an F with a segment attached (figure 18, c). As already noted, the progenies of such plants in succeeding generations consist of *normal*, *long-flowers*, and *fluted*. This result depends upon the kinds of gametes produced by the mechanism just described. *Super-long-flowers* occur in progenies of *deformed* and particularly in derived *long-flowers* lines. They are found to represent the tetra-F condition resulting from union of gametes containing two attached F's or an F with an attached segment, or two detached F's (figures 9, c, f; 19, and 20).

The appearance of *mammoth* in this complicated situation is explicable, since the F complex is concerned in the spontaneous appearance of this type (CLAUSEN 1931). Its genetic behavior is that of a monogenic recessive, but it is possibly a product of structural chromosome alteration (DARLINGTON 1932, p. 242). In untreated lines, *fluted-mammoth* and *long-flowers-mammoth* have been derived from *mammoth* as a result of the occasional production of haplo- and triplo-F types associated with the presence of the alteration which gives *mammoth*. On the other hand, the occurrence in X-ray derivatives of *deformed-mammoth*, *deformed-fluted-mammoth*, and *deformed-long-flowers-mammoth*, calls for further comment. Their occurrence is involved in the following considerations, which also apply to the occurrence of *deformed-fluted* and *deformed-long-flowers*.

All of these types which have the *deformed* character in common probably represent products of breakages of the attachment occurring in the *deformed* parents (figure 9). Of course, *deformed-mammoth* and *deformed-long-flowers-mammoth* might be related to the transmission of the two F's in their attached condition, if the alteration responsible for the production of the *mammoth* condition had occurred in all the F's, or in all except those which are deficient for the non-*mammoth* allelomorph. It seems more probable, however, that the *deformed* condition in most of these particular derivative combinations is due to the presence of unattached F's whose structural alterations are such as to induce somatic elimination. For example, 32.207, the progeny of a *deformed*, possessed a large number of *deformed-fluted* (figure 6), a phenotype which had occurred in the case of only one plant previously. Apparently, here, the attached F's seen in the

parent (31.205P35) must have been detached to give F's genetically close to normal but structurally so modified that their reproduction in somatic mitoses is impaired and leads to their occasional elimination from certain cells, which are therefore nullo-F and inviable. *Deformed-long-flowers* do not show the extent of tissue abnormality which is characteristic of *deformed-fluted*, and this indicates that the complete elimination of the F's is not as frequently accomplished in the former case. In such plants two units involving the F attachment complex must be sufficiently abnormal in structure to induce their elimination (figure 9, e). One of these units is in some cases the F with a segment attached, as already described and shown in figures 14, and 18, c, where the attached segment is responsible for the *long-flowers* type. The other F in this case may be equivalent to

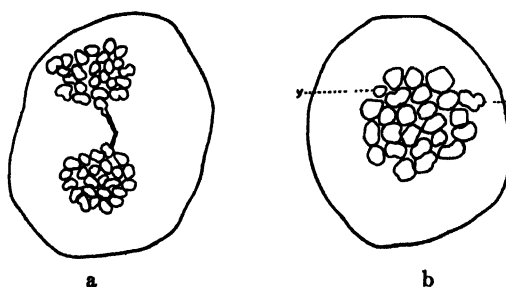


FIGURE 22.—Chromosome attachment, and one of its products: a, IIM in a plant showing *carmine-coral* variegation (31.200P8), 24 chromosomes in each plate, one of which is attached to a chromosome in the other plate; b, IM in *fluted* variant (32.200P46), derived from 31.200P8, 23II, an F chromosome with a segment attached ("x"), and a fragment ("y"). (a, Aceto-carmine;  $\times 1140$ . b, Aceto-carmine;  $\times 1100$ .)

that in *deformed-fluted*. In the case of *deformed-mammoth*, therefore, there may be two structurally altered but unattached F's by which the *mammoth* character is transmitted. The modified F present in a *fluted-mammoth* is shown in figure 21. In what way the structural modification that is evident in this case is related to the production of the *mammoth* character is not clear from the evidence available. It is clear, however, that the F chromosome shows structural alterations in all types in which the *deformed* condition is expressed morphologically.

*Carmine-coral* variegation may be related to chromosome attachment, as is shown in figure 22, a. Here the chromatin bridge connecting IIM plates is seen in a plant of this type. The chromosome constitution of a *fluted* variant derived from this plant is shown in figure 22, b. Here, in addition to 23II, there is a fragment and a univalent F chromosome which has an attached segment, both derived from the breaking of the chromatin bridge in the parent plant.

Of the many products of attached F's observed, the structurally modi-

fied F chromosome present in an  $X_3$  *fluted* deserves mention. At IM in PMC of this plant, the unpaired F chromosome could be seen among the  $23_{II}$  as a lightly-staining, deeply constricted unit somewhat larger than the normal F. Further study indicates that it is an F with a small attached segment. Such an F frequently lags at IA, and divides in the plasma at IIM. At IIT the products of this equational division are very clear because of their isolation in the cell (figure 23). It was sometimes found that in dividing, one daughter-half of the attached segment became detached (figure 23, a), so that at IIT there was one daughter-F with the attached segment and another without the attached segment, the latter being free

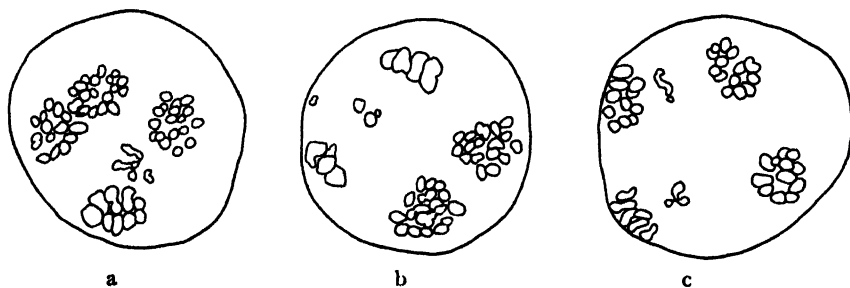


FIGURE 23.—IIT in *fluted* (30.113P14), showing division of the F chromosome with a segment attached, to give one daughter F with the attached segment, one without an attachment, and the detached segment free in the plasma. (Aceto-carmine;  $\times 1140$ .)

in the plasma (figure 23, b, c). This loss of the attached segment in a division corresponding to a somatic one suggests that a similar process may be operating in the production of "mottled" *Drosophila* described by PATTERSON (1932a). He has shown that "mottled" is the product of an unstable translocation, and has postulated that "it is possible for the chromosome with the attached fragment to split in such a manner that one cell will receive a daughter chromosome bearing the undivided fragment, while the sister cell will receive the other daughter chromosome without the fragment." The mechanism here illustrated, that is, the loss of one product of division of the fragment, would account equally well for PATTERSON'S results.

#### DISCUSSION

The evidence, genetic and cytological, concerning the "deformed assemblage" which has been presented above demonstrates the far reaching consequences of chromosome attachment of the type obtaining in this instance. The attachment concerned here has been productive of (1) races transmitting quantitative chromosomal variations (the monosomic, trisomic and tetrasomic conditions); (2) pure-breeding races transmitting recessive character contrasts which may be, however, products of structural chromosome alteration (*coral* and *mammoth* derivatives); (3) "eversport-



ing" races, products of mosaicism (*deformed*), transmitting the "ever-sporting" tendency.

We have shown that the *deformed* condition is a reflection of somatic elimination of F chromosomes as a result of a physical attachment in which they are involved. In addition to confirmatory cytological evidence, the presence of equal numbers of monosomic and trisomic types in progenies of *deformed* indicates the behavior in meiosis of this product of attachment. The genetic behavior of other elements of the *deformed* assemblage depends upon the initial presence of the attachment together with products of its breakage. These products may be, structurally, more simple and give relative simplicity in genetic behavior, as in *fluted* races derived from *deformed*. They may, however, be complex as is evidenced by the behavior of derived *long-flowers* lines, for example.

Those instances in which the genetic behavior of a *deformed* derivative cannot readily be related to products of the attachment which determined its phenotype, are interpretable in terms of secondary structural alteration or of elimination of products of the initial chromosome reorganization involved. Thus, the F chromosomes in *deformed-fluted* (30.113P15) which gave all *mammoth* suffered alteration in mitosis, while the added F responsible for the production of *deformed-long-flowers* (30.115P31) was eliminated in somatogenesis with the result that the progeny contained no *long-flowers*.

The capacity of high frequency radiation to induce quantitative chromosomal alterations has been recognized for some time. One of the first chromosomal variants thus induced, the cytogenetics of which was fully reported, was *Nubbin*, a compound chromosome derivative in *Datura* (BLAKESLEE 1927). It gave five morphologically distinct, variant types, one of which was *Nubbin*, in whose progeny the same complex segregation occurred. Thus, in genetic behavior *Nubbin* suggests *deformed*. However, all five of the variant types produced by *Nubbin* are trisomics, two being primary trisomics, two tertiary trisomics, and one *Nubbin* like the parent. *Deformed*, on the other hand, is not itself a trisomic, and its derivatives in addition to *deformed*, are monosomic, trisomic and pure-breeding recessive disomic for the F chromosome.

As already noted, *deformed* is comparable to STEIN's (1926) "farb- und-formdefekt" strain of *Antirrhinum* in external morphology and in the nature of the tissue degeneration involved. In particular her "Phytocarcinome" (PCa) R1053 (STEIN 1930), induced by radium treatment of an embryo, is of interest here. (STEIN designates her radium treated generation "R," and the progeny derived from it "R<sub>1</sub>," while we use R<sub>1</sub> and R<sub>2</sub> respectively.) The derivatives of R1053 are reminiscent of those from *deformed* so far as the complexity of types and their interrelations are con-

cerned. STEIN (1932) has shown that her case may behave as a Mendelian recessive, breeding true and segregating in  $F_2$  in crosses with normal. She, also, finds evidence of mitotic and meiotic abnormalities which she interprets as induced by the tissue degeneration produced by a recessive gene. On the other hand, we have shown that *deformed* is not the product of an induced recessive gene mutation and, further, that the mitotic and meiotic abnormalities it shows are the cause and not the effect of tissue degeneration.

The manner in which lethal deficiency effects operate in the production of the *deformed* condition calls for comment. PATTERSON (1932b) has shown that the loss from one of the X chromosomes in the zygote of *Drosophila*, of a small "viability region," possibly a single gene, has the effect of a dominant lethal. When lost from a cell during somatogenesis the cell descendants, in combination with non-deficient cells, give somatic tissues exhibiting mosaicism. In *deformed*, something of the nature of such a "viability region" is concerned but here it appears to have a recessive, in place of a dominant, lethal effect. In this connection, it is to be noted that in STEIN'S PCa individuals and, also, in the case of *deformed*, the lethal effect apparently is not strictly limited to cells deficient for the "viability region" but extends its influence to neighboring cells.

Doubtless many cases involving the occurrence of gynandromorphs, mosaics, mottles, and variegated or "eversporting" individuals are related to somatic elimination of chromosomes which have become structurally abnormal. In *Drosophila*, L. V. MORGAN (1929) found a line possessing an abnormally shaped X (the "U" line). It gave a high percentage of gynandromorphs in which it could be shown that the abnormal X was eliminated in mitosis. This chromosome was originally one of the two attached X's and doubtless its structural abnormality was attained during detachment. In this regard it is comparable to certain abnormal F chromosomes in derivatives of *deformed*.

In many features the mottled-eyed *Drosophila* case as reported by PATTERSON (1932a) resembles that of *deformed*. Both are products of X-ray treatment and in certain respects are comparable as to the morphological expression involved, genetic behavior, and, also, in respect to cytological interpretations offered. The occurrence among mottled-notched females of "individuals that show every degree of mottled and notched conditions" is paralleled by the variability of expression of the *deformed* condition and of *carmine-coral* variegation. Again, hyperploid *long-flowers* derivatives of *deformed* produce *deformed* offspring, and similarly PATTERSON reports that "fertile hyperploid flies really constitute a mottled line, from which a stock can be established." Finally, the interpretation of both cases involves the elimination of chromosome material during mitosis. On

the other hand, mottled involved an "unstable translocation"—a small segment of the X chromosome translocated to the fourth chromosome. In *deformed*, however, more than a whole chromosome is subject to elimination. It may vary in its constitution as it occurs in different individuals and in succeeding generations of *deformed*, but so long as it is subject to loss, this chromosome is seen to differ morphologically and structurally from its condition in *control*. (MULLER [1930] has noted that all cases of mottled involve some type of gene rearrangement—deletion, translocation, or inversion.)

Attention has been called above to certain reported instances in which treatment with high frequency radiation has produced chromosomal variants exhibiting complex interrelationships, tissue degeneration which is hereditary, and mosaicism involving chromosome elimination. The data submitted here as to the cytogenetics of the "*deformed* assemblage" indicate that a unique situation obtains in this case since all of these various phenomena are concerned and are capable of a consistent genetic and cytological interpretation on the basis of chromosome attachment and resulting elimination.

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# BIONOMIC STUDIES ON CERTAIN TELEOSTS (POECILIINAE).

## III. HEREDITARY BEHAVIOR OF THE COLOR CHARACTER, GOLD

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In an earlier paper (BELLAMY 1928), reference was made to a "golden" variety of *Platypoecilus maculatus* GÜNTHER. This report presents data on the hereditary behavior of this character and discusses its relation to the characters previously studied and to the work of FRASER and GORDON (1929) on the same variety. The data, all of which, except for a few matings summarized in table 1, are presented in pedigree form, table 6. They were obtained, in part, in the Hull Zoological Laboratories at the UNIVERSITY OF CHICAGO. Through 1926-1929, work progressed slowly in an improvised laboratory at my home. In 1929 facilities became available for resuming work on the new campus of the UNIVERSITY OF CALIFORNIA at Los Angeles. After many interruptions it has been possible to conclude certain analyses, results of which I wished to study before submitting the results obtained in 1923-1924.

GORDON (1927, 1931) and FRASER and GORDON (1929), have furnished excellent descriptions of the variety known as Gold. It is described as a clear transparent yellow, slightly tinged with pale orange, eyes greenish blue, dorsal fin flushed with red. GORDON finds that the color of the body compares favorably with Deep Colonial Buff, Ridgway Plate 30; dorsal fin of male, Scarlet; and dorsal fin of the female, La France Pink, Ridgway Plate 1. Eyes are Turquoise Green, Ridgway Plate 7. GORDON (1931 pp. 749, 750) notes further that this variety is without spots (macro-melanophores) and stipples (micro-melanophores) with the exception of occasional micro-melanophores in the mid-dorsal region and along the caudal peduncle. The red of the dorsal fin, in my material, often extends down onto the body, occasionally reaching the mid-ventral line near the anal and pelvic fins. In extreme cases erythrophores are present along the caudal peduncle. In the presence of the gene, or genes, for Blue (See GORDON 1931, p. 763 for description) and where the guanophores cover an extensive area the Gold variety takes on a decided silvery cast, which under certain lighting conditions, may require the use of a microscope to effect certain distinction of a few individuals from typical Blue stippled specimens.

Other characters of *Platypoecilus*, the hereditary behavior of which has been described, fall into what has been interpreted as a sex-linked multiple allelomorph series. GORDON (1926, 1927) and FRASER and GORDON (1929)

state, however, that the type described as White (BELLAMY 1928) or stipples (GORDON) is not sex-linked but autosomal. This conclusion is natural enough since the micro-melanophore pattern behaves as a simple Mendelian dominant to Gold which is certainly autosomal. As far as one can judge from the available information our differences in opinion or usage appear to be largely rhetorical, since the hereditary behavior of the stipple pattern is readily and consistently described by considering it a wild-type as is done in *Drosophila*. Geneticists find no particular difficulty in regarding red eye color in *Drosophila* as either autosomal or heterosomal, depending upon the particular allelomorph under consideration.

In describing genotypes the following symbols are used:

1.  $+$  = Wild-type (White, Stipples). The same symbol,  $+$ , is also used for the wild-type allelomorph of  $N$ ,  $P$ , and  $R$ .
2.  $g$  = Gold.
3.  $N$  = Nigra. (Stippled. Macro-melanophores arranged in a definite pattern.)
4.  $R$  = Rubra. (Stippled, red, spotted. While females are usually, but not always, much less red than males, they are more variable and develop the red color later.)
5.  $P$  = Pulchra. (Stippled, spotted, non-red.)

A Nigra-Gold specimen is non-stippled and shows typical effects of the gene  $g$ , except in those regions of the body that are obscured by the Nigra pattern. Pulchra-Gold is non-red, non-stippled, spotted, but otherwise showing typical effects of  $g$ . Rubra-Gold is red, spotted, non-stippled. Separation of Rubra-Gold from Rubra is most easily effected by isolating all Gold young soon after birth, from matings where both phenotypes are expected. The separation can be made in adult fish but it is necessary to use a microscope on many specimens to determine the presence or absence of micro-melanophores.

It will be convenient to use any of the above symbols, except  $g$  in place of the  $X$  (or  $Z$ ) chromosome symbol, and  $O$  for the  $Y$  (or  $W$ ) chromosome symbol. Heterosomic or sex-linked characters (genes) are written first. Thus,  $RR\ gg$  and  $RO\ gg$  represent Rubra-Gold male and female respectively.  $++\ gg$  represents a Gold male;  $+Og+$ , a female heterozygous for the wild-type allelomorph of Gold and with the heterosomal wild-type allelomorph of the multiple allelomorphs,  $R$ ,  $N$ , or  $P$ . In this manner the necessity for giving the wild-type symbol a special subscript label is avoided.

The pedigree, table 6, is so arranged that the reader may compare the data with any hypothesis. Since original record numbers are used it is really a continuation of the pedigree previously published (BELLAMY 1928,

table 3). The type of mating is indicated in the first column where the genotype of the male parent is written first. The second column gives the original record number; third and fourth columns the source of the parents. Where the source of material is unknown, usually obtained from a local dealer, the fact is indicated by the letter *U*. In a few cases material has been taken from unpedigreed stocks kept for varying lengths of time in the laboratory. The number of young born is recorded in the fifth column and in the sixth to twelfth columns the phenotype of offspring. Males are recorded above and females in the line below as indicated for the first mating of the table. Where the color of young has been recorded soon after birth the number is recorded in parentheses under the phenotype they most closely resemble.

Crosses of Gold with the Wild-type were made reciprocally with substantially identical results, (table 1).

TABLE 1

		Gold <i>gg</i>	×	Wild-type <i>++</i>	
		Wild-type <i>g+</i>			
<i>F</i> <sub>1</sub>		♂ 110		♀ 129	(Matings 303, 322, 462, 465)
Wild-type <i>++</i> and <i>g+</i>		Gold <i>gg</i>			
♂ 124	♀ 144	♂ 37		♀ 25	(Matings 303.1, 322.1, 450.2, 462.1, 463.1)

Aside from the relatively small number of *F*<sub>2</sub> Golds, which is probably insignificant with such small numbers, the chief matter for comment is the appearance of *F*<sub>1</sub> and *F*<sub>2</sub> Wild-types. They approximate the Wild-type in general appearance, but dominance is not complete except as regards stippling. The dorsal fin is distinctly tinged with red, most marked near the base of the fin, and in many specimens, mostly males, there is a trace of red color on the abdominal wall near the anal and pelvic fins. The red color in the dorsal fin is more conspicuous in males than in females. This tendency toward an intermediate condition indicates that Gold is not simply a fish without stipples but that it has certain positive attributes of its own.

Since *P*, *N*, and *R* may be regarded as belonging to the same allelomorph series, crosses involving these three patterns and Gold are tabulated together. The record numbers of the matings included in the tabulation, table 2, are given and the reader may study each cross separately by referring to the pedigree, table 6. The letter *C* is used in tables 2 and 4 to replace *P*, or *R*, or *N*.

Separation of Rubra-Gold from Rubra was not considered accurate enough in mating 304.1, the first in which these types appeared, to justify inclusion in the tabulation. Results of this mating are included, however, in table 3.

TABLE 2

		Gold ♂ ++gg		× Colored ♀ CO++			
F <sub>1</sub>		Colored ♂♂ C+g+		Wild-type ♀♀ +Og+		(Matings 304, 305, 306)	
F <sub>2</sub>	Colored		Wild-type		Colored-Gold		Gold
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	
	65	70	63	86	9	9	14
							23
	(Matings 305.1, 305.2, 306.1, 306.2)						

TABLE 3

	SPOTTED		RED		NON-SPOTTED		SPOTTED		NON-RED	
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
F <sub>1</sub>	48	0	0	0	0	0	0	0	0	54
F <sub>2</sub>	91	106	0	0	0	0	0	0	91	119

The results given in table 2 may be compared, to some extent, with the data of FRASER and GORDON (1929, table 2) by reclassifying the data and using their descriptive names (table 3).

Data from reciprocal crosses, Colored male × Gold female, are presented in table 4, and compared with similar data of FRASER and GORDON (1929, table 1) in table 5.

TABLE 4

		Colored ♂ CC++		× Gold ♀ ++gg			
Colored		Wild-type (inter-sex ?)					
♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
C+g+	COg+						
89	54			1			
							(Matings 317, 388, 389, 390)
Colored		Wild-type		Colored-Gold		Gold	
♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
96	57	0	49	27	17	0	17
							(Matings 388.1, 388.2, 389.1, 390.1)



TABLE 5

	RED				NON-RED			
	SPOTTED		NON-SPOTTED		SPOTTED		NON-SPOTTED	
	♂ ♂	♀ ♀	♂ ♂	♀ ♀	♂ ♂	♀ ♀	♂ ♂	♀ ♀
F <sub>1</sub>	89	54	0	0	0	0	0	1 0
F <sub>2</sub>	123	74	0	0	0	0	0	66

It will be noted that only matings 304, 304.1, 317, 389, and 389.1 are directly comparable with the results of FRASER and GORDON. The other types of matings, however, involving *N* and *P* are better suited to the analysis in question since, with one exception, all of the phenotypes are easily distinguished and many of the genotypes can be determined by inspection. The distinction between Rubra-Gold and Rubra is mentioned on page 523. By isolating all of the Gold young in mating 389.1 soon after birth the separation of the two phenotypes was easily effected.

Unfortunately a detailed comparison between these data and those of FRASER and GORDON (1929) is not possible, since apparently they have thrown certain phenotypes together. In the headings to their tables 1, 2, 3, and 4, appears the phrase "Gold—(non-spotted, non-red)" and while it is legitimate to infer that the offspring labeled in the tables as "non-red, non-spotted" are Gold, it seems probable that they have simply suppressed certain details for the sake of brevity or to emphasize a particular point. In all of the F<sub>2</sub> matings in tables 2 and 4 above and presumably in FRASER and GORDON's tables 1 and 2, among others, all colored spotted animals fall into two phenotypes, namely, Gold and not Gold. Likewise the non-spotted F<sub>2</sub> offspring are of two types, Gold and not Gold.

As far as most of my own data go, *R*, *N*, and *P* are satisfactorily treated as "unit characters" depending upon a difference in one pair of genes. The appearance of a non-spotted red male and an "exceptional" red, spotted female in FRASER and GORDON's laboratory makes it necessary to re-examine the question of the genetic constitution not only of Rubra but also of Nigra and Pulchra. Discussion of this matter follows in the fourth report of this series (BELLAMY 1933) on linkage and non-disjunction in *Platypocilus*.

#### SUMMARY

1. The hereditary behavior of Gold, an autosomal character, in relation to the multiple allelomorphic series, *N*, *P*, *R*, and the Wild-type, are described and compared with FRASER and GORDON's (1929) crosses between Rubra and Gold.

2. Gold behaves as a simple Mendelian recessive to the Wild-type. Dominance of the Wild-type is not complete.

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TABLE 6

MATING	RECORD NUMBER	MALE	SOURCE OF FEMALES	YOUNG BORN	GOLD	WILD- TYPE	NIGRA	NIGRA- GOLD	RUBRA	RUBRA- GOLD	PULCHRA	PULCHRA- GOLD	SEX NOT RECORDED
<i>gg</i> × <i>gg</i>	302	<i>U</i>	<i>U</i>	71	♂ 29 ♀ 30	..	..	..	..	..	..	..	12
<i>gg</i> × <i>gg</i>	302.1	302	302	136	29 30	..	..	..	..	..	..	..	77
<i>gg</i> ×++	303	302	260	98	.. ..	38 22	..	..	..	..	..	..	38
<i>g</i> × <i>g</i> +	303.1	303 <sub>1</sub>	303	177	(47) 10 2	(130) 23 22	..	..	..	..	..	..	120
++ <i>gg</i> × <i>RO</i> ++	304	302 <sub>1</sub>	44.3.2	102	.. .	.. 9	..	..	11	..	..	..	82
<i>R</i> + <i>g</i> ×++ <i>Og</i> +	304.1	304	304	147	(31) 3 4	(101) 14 20	..	..	17 27	..	..	..	62
++ <i>gg</i> × <i>NO</i> ++	305	302 <sub>1</sub>	217.2	70	.. .	.. 22	23 ..	..	..	..	..	..	25
<i>N</i> + <i>g</i> ×++ <i>Og</i> +	305.1	305	305	49	(14) 0 4	(35) 1 1	5 .. 5	0 .. 2	..	..	..	..	31
<i>N</i> + <i>g</i> ×++ <i>Og</i> +	305.2	305	305	140	(27) 7 2	(100) 16 21	20 12 ..	4 2 ..	..	..	..	..	56
++ <i>gg</i> × <i>PO</i> ++	306	302 <sub>1</sub>	83.1.2	40	.. ..	.. 17	.. ..	..	..	..	20	..	3

TABLE 6 (Continued)

MATING	RECORDED NUMBER	SOURCE OF MALE	SOURCE OF FEMALE	YOUNG BORN	GOLD	WILDA- TYPE	NIGRA	NIGRA- GOLD	RUBRA	RUBRA- GOLD	FULCERA	FULCERA- GOLD	SEX NOT RECORDED
$P+g++X+Og++$	306.1	306	306	228	(41) 6 5	(187) 26 40	..	..	..	..	27 39	4 3	78
$P+g++X+Og++$	306.2	306	306	95	(10) 2 2	(73) 20 24	..	..	..	..	13 14	1 2	17
$gg \times gg$	311	<i>U</i>	<i>U</i>	67	25 14	..	..	..	..	..	..	..	28
$RR++X+Ogg$	317	44.2.1	311	157	..	1	..	..	40	..	..	..	69
$++ \times gg$	322	117.1.1	302	93	..	25 35	..	..	..	..	..	..	33
$g \times g+$	322.1	322	322	56	(17) 13 2	(39) 18 20	..	..	..	..	..	..	3
$P++++X+Ogg$	324	263	311	66	..	4 9	..	..	..	..	12 13	..	28
$N++++X+Ogg$	325	265	311	6	..	1 1	1 1	..	..	..	..	..	2
$++gg \times NOgg$	357	302	305.1	13	..	..	..	8	..	..	..	..	0
$N++++X+Ogg$	358	305.1	311	37	..	11 8	5 7	..	..	..	..	..	6
$N+g++X+Ogg$	359	305.1	311	14	1 0	6 5	0 1	1 0	..	..	..	..	0

TABLE 6 (Continued)

MATING	RECORD NUMBER	SOURCE OF MALE	SOURCE OF FEMALE	YOUNG BORN	GOLD	WILD- TYPE	NIGRA	NIGRA- GOLD	RUBRA	RUBRA- GOLD	FULCHRA	FULCHRA- GOLD	SEX NOT RECORDED
<i>R+g+X+O++</i>	360	304	314	70	..	7	..	..	11	..	..	..	25
					..	9	..	..	18	..	..	..	
<i>R+g+X+Ogg</i>	361	304	302	59	(22)	(37)							
					3	7	..	..	6	4	..	..	15
					5	9	..	..	8	2	..	..	
<i>PP++X+Ogg</i>	388	Stock	Stock	60	..	..	..	..	..	..	19	..	17
					..	..	..	..	..	..	24	..	
<i>P+g+XPOg+</i>	388.1	388	388	47	(10)	(37)							
					..	..	..	..	..	..	14	2	10
					2	7	..	..	..	..	9	3	
<i>P+g+XPOg+</i>	388.2	388	388	104	(25)	(79)							
					..	..	..	..	..	..	32	10	19
					5	14	..	..	..	..	18	6	
<i>RR++X+Ogg</i>	389	Stock	Stock	38	..	..	..	..	8	..	..	..	23
					..	..	..	..	7	..	..	..	
<i>R+g+XROg+</i>	389.1	389	389	73	(16)	(57)							
					..	..	..	..	17	4	..	..	26
					3	10	..	..	10	3	..	..	
<i>NN++X+Ogg</i>	390	Stock	Stock	52	..	..	22	..	..	..	..	..	8
					..	..	22	..	..	..	..	..	
<i>N+g+XNOg+</i>	390.1	390	390	114	(29)	(85)							
					..	..	33	11	..	..	..	..	20
					7	18	20	5	..	..	..	..	

BIONOMIC STUDIES ON CERTAIN TELEOSTS  
(POECILIINAE). IV. CROSSING OVER AND  
NON-DISJUNCTION IN *PLATYPOECILUS*  
*MACULATUS* GÜNTHER

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In previous reports (BELLAMY 1928, 1933) the characters Rubra, Nigra, and Pulchra have been interpreted as gene mutations at a single locus on one of the heterosomes, although the possibility of close linkage was recognized (BELLAMY 1928, p. 229). Data presented by FRASER and GORDON (1929) indicate that Rubra, supposing that the Rubras in their laboratory and mine are of the same sort, very probably is a composite of the characters "Red" and "Spots." They observe the appearance of a red, non-spotted fish, which, because of its breeding behavior, was interpreted by them as due probably to crossing over between X and Y chromosomes. The separation of Red and Spots has not been observed in my laboratory but the appearance of certain other exceptional individuals favors the interpretation of crossing over between the X and Y chromosomes in this species.

Incidentally it is probable that it was the interpretation of the origin of this non-spotted red that led GORDON (1931, p. 764) so completely to misinterpret one of my statements (BELLAMY 1928) relative to the simultaneous occurrence of the Rubra and Pulchra patterns in females. Apparently GORDON assumes the Pulchra to be identical with Rubra minus the gene for red color. It is possible of course that Pulchra had its origin from the Rubra-type but I know of no direct evidence on that point. I do know, however, that the Rubra-Pulchra pattern has not been observed in my aquaria in females, and that a Rubra-Pulchra male (red and spotted) is fairly easily distinguished from Rubra (also red and spotted) in mature specimens. The occurrence of red, spotted (Rubra) females was described in my first report (BELLAMY 1924) and mentioned in so many places, in addition to detailed descriptions of their hereditary behavior, in the second report (BELLAMY 1928) that GORDON's statement that "BELLAMY (1928) has taken the curious position of doubting the existence of such a color combination (Rubra or red spotted platy) in the female," is just a little ludicrous.

The occurrence of non-spotted red as described by FRASER and GORDON raises the question of the genetic constitution of both Pulchra and Nigra in addition to Rubra. It is possible that they are composite patterns also but until they are resolved recognition is impossible.

If we suppose that Red and Spots had separate origins, Rubra arose, presumably from crossing over between two heterosomes. In this case Pulchra can be regarded as a Rubra less the gene *R*, FRASER and GORDON's symbol *Sp* can be used for it, and a chromosome map represented as in figure 1. Stipples or White, is regarded as the wild-type and its symbol, +, is not included.

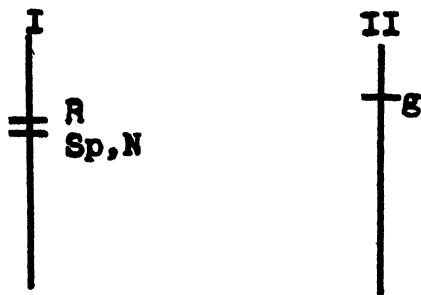


FIGURE 1

A cross of Pulchra male  $\times$  Rubra female would then be written, following the *Drosophila* schema:  $\frac{+Sp}{+Sp} \times \frac{RSp}{+Sp}$ , and the fish described as a Rubra-

Pulchra would be:  $\frac{RSp}{+Sp}$ . The + symbol is used here in the same sense as FRASER and GORDON's recessive *sp*.

A possible method for obtaining additional information, in the absence of additional crossing over, involves matings between Red, non-spotted and Pulchra (figure 2).

$$\text{FIGURE 2.} \text{—Red, non-spotted } \frac{R+}{R+} \times \frac{+Sp}{+Sp} \text{ Non-red, spotted (Pulchra)}$$

$$\text{Red, spotted } \frac{R+}{+Sp} \quad \frac{R+}{+Sp} \quad \text{Red, non-spotted}$$

The red, spotted males,  $\frac{R+}{+Sp}$  should be substantially identical with typical Rubras and therefore distinguishable from the typical Rubra-Pulchra males obtained from crosses of Rubra males and Pulchra females. As previously noted Pulchra-Rubra males have a more extended pattern of macro-melanophores than typical Rubras. The spots tend to be larger and are more numerous.

The next question involves the relation of Nigra to Red, *R*, and Spots, *Sp*. *N* might be regarded as a pattern factor complementary to *Sp* or simply as allelomorphic to *R*.

If  $N$  is allelomorphic to  $S\phi$ , and is a complete unit in itself, sooner or later crossing over will effect a relatively stable combination of  $R$  and  $N$ . Such a fish, if encountered in nature, in the absence of special information would be regarded as a single unit as was the case with Rubra before FRASER and GORDON observed the appearance of the non-spotted red fish. If  $N$  is complementary to  $S\phi$ , crossing over would result in  $N$  and  $R$  genes entering one gamete, + and  $S\phi$ , another, the latter fertilizing an egg containing the Y chromosome of the female, leaving the vacant (as regards  $N$  and  $S\phi$ ) X chromosome to be fertilized by an X bearing sperm carrying the wild-type gene.

If  $N$  is allelomorphic to  $R$  and crossing over occurs in the female between the X and Y chromosomes, a separation of  $N$  from  $S\phi$  can result, and the event would be similar to the separation of  $R$  from  $S\phi$  as assumed by FRASER and GORDON (1929, p. 171). The other crossover described by FRASER and GORDON (1929, pp. 168 *et seq.*) differs in that both  $R$  and  $S\phi$  are supposed to have entered the Y chromosome together.

Crossing over of the type indicated above can explain the appearance of the exceptional Wild-type female recorded in mating 89, table 3 (BELLAMY 1928). Since the Nigra pattern results from a definite organization of macro-melanophores, presumably a fish having the gene for  $N$  but not for  $S\phi$  would be a reversion to the wild-type. The appearance of this exceptional female is accounted for, however, equally well by assuming that both  $N$  and  $S\phi$  entered the Y chromosome of the female, as indicated above. Likewise the three exceptional Wild-type females appearing in mating 44.2.1, table 3, (BELLAMY 1928) are accountable for in a similar manner. If this is crossing over, it has occurred four times among 962 offspring in matings such that detection was possible. This amounts to approximately 0.4 percent. Since, in this case, only half of the crossovers are detectable, the indicated value is approximately 0.8 percent, a value that need not be taken too seriously until more data are available.

The exceptional Wild-type individual recorded in mating 317 (tables 4, 6, BELLAMY 1933) requires a different explanation. This individual was born October 20, 1923, and on March 20, 1924, it was recorded as a white (Wild-type) heterozygous for Gold. On April 15, 1924, it was isolated as an immature male since it showed the typical thickening and pseudo-segmentation of the third anal ray. On May 21, 1924, it was noted on the records that the fish had made "no further progress toward maleness. Third anal ray shows only a thickened condition with segmentation extending into distal third." Since the male parent was a homozygous Rubra, crossing over in the female cannot account for the production of a Wild-type of either sex. Non-disjunction in the male, however, and fertilization of an egg by a sperm having no X chromosome would produce a zygote having



either an X or a Y chromosome, depending upon which type of egg was involved. The fact that the fish did not develop into a normal individual of either sex further indicates an abnormal distribution of sex factors.

#### SUMMARY

1. The bearing of FRASER and GORDON's (1929) findings is discussed in relation to the genetic constitution of *Pulchra* and *Nigra*. It appears probable that *Pulchra* is identical with FRASER and GORDON's "Spots."

2. A value somewhat under one percent is indicated for crossing over between X and Y chromosomes in the female.

3. An exceptional Wild-type inter-sex (?) is interpreted as resulting from non-disjunction in the male.

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# AN EXPERIMENTAL AND THEORETICAL STUDY OF CHROMATID CROSSING OVER<sup>1</sup>

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## INTRODUCTION

The term "crossing over" is used to denote the exchange of pieces or segments between homologous chromosomes. There are many facts which indicate that the exchange of parts or segments occurs during the first meiotic prophase when the two homologous chromosomes are in intimate association. Prior to 1916 crossing over was thought to take place between the two paired chromosomes before they had divided equationally. BRIDGES, however, in that year, from his study of non-disjunction of the X chromosome of *Drosophila melanogaster* came to the conclusion that each chromosome was split equationally when crossing over occurred. Later L. V. MORGAN (1925), ANDERSON (1925) and BRIDGES and ANDERSON (1925) in a beautiful series of experiments substantiated BRIDGES' earlier conclusion; and ANDERSON (1925) and BRIDGES and ANDERSON (1925) further demonstrated that only two of the four strands crossed over at any one level. This was true for both diploids and triploids. REDFIELD (1930, 1932) working with triploid *Drosophila* found that the II and III chromosomes likewise crossed over when each chromosome was split equationally. ANDERSON used the phrase "four strand crossing over" to denote the divided condition of the chromosomes at the time crossing over took place in diploids. This term should not be applied to triploids, since it infers diploidy, even though only two of the three chromosomes (four chromatids) are involved in any one point of crossing over. The terms "double strand" or "chromatid crossing over" could be used for both diploids and polyploids since they refer only to the presence of the equational split.

In addition to the chromosomes of *D. melanogaster*, the X chromosomes of *D. simulans* (STURTEVANT, 1929) and *D. virilis* (DEMEREK, unpublished) have been genetically proved to cross over at a double strand stage. RHOADES (1932) presented data which proved that the *pr-v<sub>2</sub>* chromosome in *Zea*, the fifth largest in the monoploid complement, crossed over after, or at the time, the equational split occurred. WHITING and GILMORE (1932) reached a similar conclusion for one chromosome of *Habrobracon* in their study of impaternal daughters from virgin females. As far as the writer is aware, these are the only cases where chromatid or double strand crossing

<sup>1</sup> Paper No. 194 from the Department of Plant Breeding, Cornell University, Ithaca, New York.

over has been genetically demonstrated. (LINDEGREN recently [1933] demonstrated chromatid crossing over in the fungus *Neurospora*.) There are certain data (BLAKESLEE and others 1923, FROST 1931) in plants which can be interpreted as the result of double strand crossing over, but since other explanations are possible they can not be said to prove that double strand crossing over occurred.

In recent years many cytological papers by various investigators have dealt with the nature of the tetrad present in the first meiotic division of diploid organisms. They have attempted to discover the cytological mechanism by which genetic crossing over is effected through their study of the nature and origin of chiasmata. There is much controversy regarding many salient points, but most of the investigators agree that only two of the four chromatids in each chiasma are involved in the actual exchange of partners.

STERN (1931) and CREIGHTON and McCLINTOCK (1931) have proved that genetic crossing over is accompanied by an actual exchange of parts between the chromosomes. Later (1932) CREIGHTON and McCLINTOCK presented cytological evidence that crossing over in *Zea* takes place between chromatids. *Zea* thus becomes the first organism in which chromatid crossing over has been demonstrated both genetically and cytologically.

The occurrence of chromatid crossing over in such diverse forms as *Drosophila*, *Habrobracon*, and maize suggests that it may be a wide spread or universal phenomenon. However, WETTSTEIN's data on *Funaria*, where he found only two types of spores among the quartets from sporophytes which were heterozygous for linked factors, indicates that crossing over in *Funaria* takes place between undivided chromosomes and not between chromatids.

#### DOUBLE STRAND CROSSING OVER IN *ZEA*

In diploid organisms where the four chromatids which comprise the tetrad are normally distributed to the quartet of cells arising from each meiocyte and where it is impossible to recover the four resulting cells from any given meiocyte it is impossible to tell genetically whether crossing over occurs in a single or double strand condition. It was only through such aberrant behavior as non-disjunction which results in two of the four chromatids going to a single member of the quartet that the occurrence of double strand crossing over was genetically proven in diploid *Drosophila*. In trisomic and polyploid individuals where the number of homologous chromosomes present in metaphase I makes it possible for some members of the quartet to regularly receive more than one chromosome it is possible to test for the occurrence of double strand crossing over.

For this study in *Zea* the *pr-v*<sub>2</sub> trisome, which involves the fifth largest

chromosome, was used since these trisomic plants differ markedly in appearance from their disomic sibs and an accurate classification into the two classes is possible.

The writer, in 1932, published a preliminary note on the genetical demonstration of double strand crossing over in *Zea*. This paper will present more extensive data upon this subject.

The factor pair *Pr:pr* differentiates between purple and red aleurone color and the factor pair *V<sub>2</sub>:v<sub>2</sub>* is responsible for green and virescent seedling color. These two pairs of genes give a recombination value of 41 percent as shown by the data in table 1. The crossover value or map distance

TABLE 1  
*Control data for percent of recombinations between pr and v<sub>2</sub> in diploids. Pr V<sub>2</sub> pr v<sub>2</sub> × pr v<sub>2</sub>.*

PEDIGREE	PURPLE ALEURONE GREEN SEEDLING	PURPLE ALEURONE VIRESCENT SEEDLING	RED ALEURONE GREEN SEEDLING	RED ALEURONE GREEN SEEDLING
1831-1840	438	267	288	375

40.6 percent of recombinations.

is much greater than this since undetected double crossovers reduce the percent of recombinations.

Trisomic plants of *Pr V<sub>2</sub>* constitution were

$$\frac{Pr}{pr} \frac{V_2}{v_2}$$

pollinated by double recessive individuals. The ensuing seeds were classified into purple and red aleurone classes and when planted the seedlings classified as green or virescent. Classification into trisomic and disomic types was made just before anthesis.

If crossing over among the members of the trivalent occurred between undivided chromosomes and not between chromatids there should be no cases of trisomic plants homozygous for *pr* or *v<sub>2</sub>*. If, however, crossing over took place between chromatids the occurrence of trisomic plants homozygous for the two loci is expected. Diagram 1 shows how a trisomic plant homozygous for *v<sub>2</sub>* may arise. Similarly if a crossover had taken place between *pr* and the spindle insertion, a trisomic homozygous for *pr* would be possible. The data in table 2 show 61 trisomic individuals homozygous for the *v<sub>2</sub>* gene. There were nine plants among the 553 individuals arising from red aleurone (*pr*) seed which were trisomic. Only two of the 15 pedigrees listed in table 2 failed to throw exceptional trisomic types.

The constitution of the trisomic plants was *AACCRr* with respect to the aleurone factors affecting color. Since the male parents were *AA*

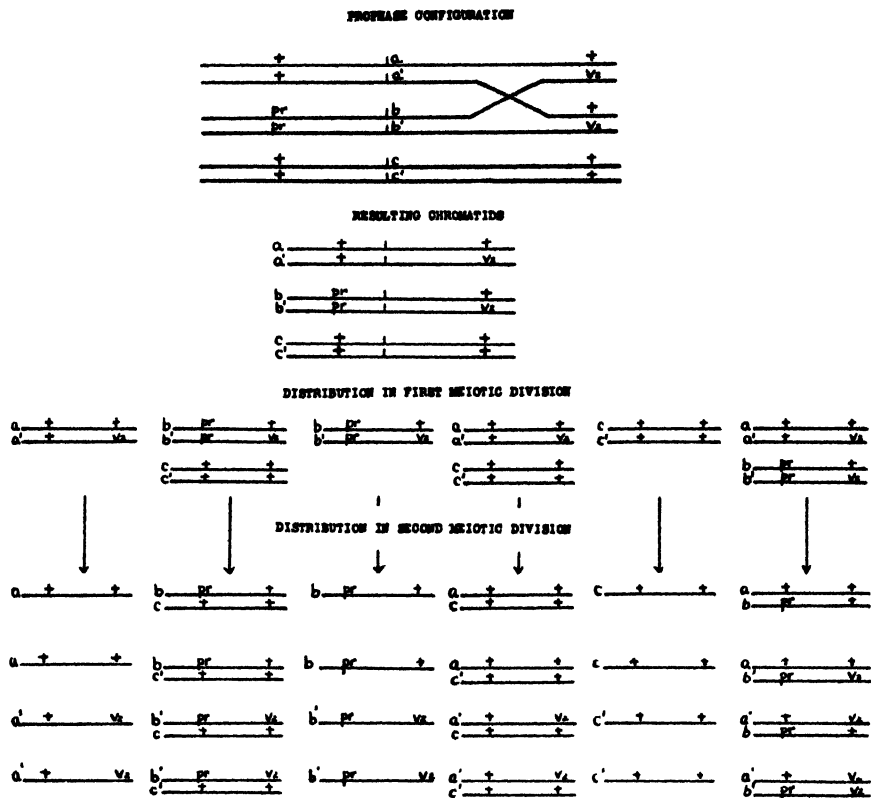


DIAGRAM I

Diagram illustrating how a trisomic plant homozygous for  $v_2$  may arise through chromatid crossing over. The combination in the lower right hand corner will give rise to the exceptional trisomic type.

*CCRR* it was expected that all the seeds would have colored aleurone and could be classified as purple or red. Surprisingly enough approximately 50 percent of the seeds had colorless aleurone. Subsequent investigation showed that two of the  $r$  genes were dominant over a single  $R$  gene. This  $r$  allelomorph has been designated  $r^{rw}$  (the superscript  $r$  to represent its effect upon plant color and the superscript  $w$  to represent its effect upon aleurone color). This behavior is analogous with the floury-flinty endosperm situation. Although the  $Pr:pr$  constitution of these colorless seeds could not be told without testing, they were planted since data on the  $v_2$  locus could be had.

Since approximately only one-half of the seeds could be classified for aleurone color the total number of trisomic plants homozygous for  $pr$  should be twice 9, or eighteen. This is a legitimate procedure since the  $R$  locus is in another chromosome. Five of the nine  $pr$  trisomes were also

TABLE 2

*Data on cross of  $\frac{Pr}{pr} \times \frac{V_2}{v_2}$  trisomes by double recessive male parents.*

PEDIGREE	CULTURE	ALBURONE	2N GREEN	2N $v_2$	2N+1 GREEN	2N+1 $v_2$
1482 (1) × 1055	1620	235 <i>Pr</i>	79	23	72	4
	1621	279 wh.	95	47	73	3
	1622	59 <i>pr</i>	24	27	0	0
1482 (2) × 1055	1623	20 <i>pr</i>	2	12	0	0
	1624	146 wh.	55	38	19	0
	1625	115 <i>Pr</i>	49	12	24	0
1482 (3) × 1055	1626	157 <i>Pr</i>	41	15	65	2
	1627	43 <i>pr</i>	15	25	0	0
	1628	132 wh.	46	32	28	3
1482 (4) × 1055	1629	40 <i>pr</i>	9	27	0	2
	1630	169 <i>Pr</i>	65	17	50	2
	1631	175 wh.	74	32	40	3
1482 (5) × 1055	1632	231 <i>Pr</i>	93	21	68	5
	1633	237 wh.	78	50	53	2
	1634	59 <i>pr</i>	20	24	0	1
1482 (6) × 1055	1635	41 <i>pr</i>	15	20	0	0
	1636	133 <i>Pr</i>	57	13	38	1
	1637	201 wh.	76	28	52	0
1482 (7) × 1055	1638	186 <i>Pr</i>	65	15	70	3
	1639	51 <i>pr</i>	16	28	0	0
	1640	171 wh.	69	36	33	3
1482 (8) × 1055	1641	185 <i>Pr</i>	65	24	43	1
	1642	65 <i>pr</i>	14	30	1	0
	1643	268 wh.	84	50	60	6
1482 (9) × 1055	1644	36 <i>pr</i>	9	23	0	1
	1645	146 wh.	50	27	48	1
	1646	124 <i>Pr</i>	44	11	50	1
1482 (10) × 1055	1647	194 <i>Pr</i>	87	19	57	3
	1648	56 <i>pr</i>	21	24	0	0
	1649	253 wh.	106	50	48	1
1482 (11) × 1055	1650	60 <i>pr</i>	12	28	2	1
	1651	228 <i>Pr</i>	62	10	66	3
	1652	281 wh.	99	46	49	1
1582 (12) × 1055	1653	34 <i>pr</i>	8	8	0	0
	1654	170 wh.	52	34	21	0
	1655	121 <i>Pr</i>	23	15	28	0
1482 (13) × 1055	1656	34 <i>pr</i>	7	20	1	0
	1657	131 <i>Pr</i>	50	12	46	1
	1658	179 wh.	55	37	55	2
1482 (14) × 1055	1659	227 <i>Pr</i>	82	27	71	1
	1660	266 wh.	116	47	50	4
	1661	64 <i>pr</i>	21	36	0	0
1482 (15) × 1055	1662	34 <i>pr</i>	12	8	0	0
	1663	111 wh.	38	14	20	0
	1664	126 <i>Pr</i>	50	4	38	0

homozygous for  $v_2$ . These individuals might have arisen through non-disjunction at any one of the four divisions following the first meiotic division. But the normal frequency of non-disjunction during these divisions is so low that this interpretation can be disregarded. The  $v_2$  trisomes from purple aleurone seed all proved to be  $\frac{Pr}{pr} \frac{v_2}{v_2}$  in constitution. The

$$\frac{\frac{pr}{pr} \quad v_2}{v_2}$$

male pronucleus brought in a single  $\frac{pr}{pr} \frac{v_2}{v_2}$  chromosome so the egg must have been  $\frac{Pr}{pr} \frac{v_2}{v_2}$  in constitution.<sup>1</sup> The origin of eggs of this type is

$$\frac{pr}{pr} \quad v_2$$

possible only through chromatid or double strand crossing over. The same argument holds for those  $pr$  trisomes of  $\frac{pr}{pr} \frac{V_2}{v_2}$  constitution. Here the

$$\frac{\frac{pr}{pr} \quad v_2}{v_2}$$

egg must have been of  $\frac{pr}{pr} \frac{V_2}{v_2}$  constitution and this combination is pos-

$$\frac{pr}{pr} \quad v_2$$

sible only through chromatid crossing over. The genotypic constitutions of all the exceptional trisomic plants are presented in table 3. This table

TABLE 3  
*Genotypic constitutions of exceptional trisomes listed in table 2.*

PLANT	GENOTYPIC CONSTITUTION	2N+1 BY ROOT TIP COUNTS	2n+1 BY ALEURONE OR SEEDLING RATIOS	2n+1 BY APPEARANCE
1620 (2)	$Pr \ pr \ pr \ v_2 \ v_2 \ v_2$	"	"	"
1620 (3)	$Pr \ pr \ pr \ v_2 \ v_2 \ v_2$	"	"	"
1620 (A)	$Pr \ pr \ pr \ v_2 \ v_2 \ v_2$	"	"	"
1620 (B)	$Pr \ pr \ pr \ v_2 \ v_2 \ v_2$	"	"	"
1621 (1)	$v_2 \ v_2 \ v_2$	"	—	"
1621 (2)	$v_2 \ v_2 \ v_2$	"	—	"
1621 (A)	$v_2 \ v_2 \ v_2$	"	—	"
1626 (A)	$Pr \ pr \ pr \ v_2 \ v_2 \ v_2$	"	"	"
1626 (1)	$Pr \ pr \ pr \ v_2 \ v_2 \ v_2$	"	"	"
1628 (1)	$Pr \ pr \ pr \ v_2 \ v_2 \ v_2$	"	"	"
1628 (2)	$Pr \ pr \ pr \ v_2 \ v_2 \ v_2$	"	"	"
1628 (3)	$Pr \ pr \ pr \ v_2 \ v_2 \ v_2$	"	"	"
1629 (1)	$pr \ pr \ pr \ v_2 \ v_2 \ v_2$	"	—	"
1629 (2)	$pr \ pr \ pr \ v_2 \ v_2 \ v_2$	"	—	"
1630 (2)	$Pr \ pr \ pr \ v_2 \ v_2 \ v_2$	"	"	"
1630 (3)	$Pr \ pr \ pr \ v_2 \ v_2 \ v_2$	"	"	"
1631 (1)	$Pr \ pr \ pr \ v_2 \ v_2 \ v_2$	"	"	"
1631 (2)	$v_2 \ v_2 \ v_2$	"	—	"
1631 (3)	$v_2 \ v_2 \ v_2$	—	—	"

<sup>1</sup> Extensive tests made by the writer show that pollen carrying an extra  $pr-v_2$  chromosome rarely, if ever, functions in competition with haploid pollen since of a total of 1845 plants from the cross of a disome by a trisome there were no trisomic plants.

TABLE 3 (Continued)

PLANT	GENOTYPIC CONSTITUTION	2N+1 BY ROOT TIP COUNTS	2n+1 BY ALBUKOVIC OR SEEDLING RATIOS	2n+1 BY APPEARANCE
1632 (1)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1632 (2)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1632 (5)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1632 (6)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1632 (7)	<i>Pr ? pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	—	"
1633 (1)	<i>pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1633 (2)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1634 (1)	<i>pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	—	"
1636 (1)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1638 (2)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1638 (A)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1638 (B)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1640 (A)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	"	"
1640 (B)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1640 (C)	<i>v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	—	"
1641 (1)	<i>Pr ? pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	"	"
1642 (2)	<i>pr pr pr V<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	"	"
1643 (3)	<i>Pr ? pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	—	"
1643 (2)	<i>v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	—	"
1643 (B)	<i>Pr ? pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	"	"
1643 (C)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1643 (D)	<i>v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	—	"
1643 (E)	<i>v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	—	"
1644 (1)	<i>pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	—	"
1645 (1)	<i>pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1646 (1)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	"	"
1647 (1)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1647 (2)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	"	"
1647 (3)	<i>Pr ? pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	—	"
1649 (1)	<i>v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	—	"
1650 (1)	<i>pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1650 (2)	<i>pr pr pr V<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1650 (3)	<i>pr pr pr V<sub>2</sub> V<sub>2</sub> v<sub>2</sub></i>	"	"	"
1651 (1)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1651 (2)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1651 (4)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1652 (1)	<i>v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	—	"
1656 (1)	<i>pr pr pr V<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1657 (2)	<i>Pr ? pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	—	"
1658 (A)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	"	"
1658 (B)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1659 (1)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1660 (A)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1660 (B)	<i>Pr pr pr v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"
1660 (C)	<i>v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	—	"
1660 (D)	<i>v<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	—	—	"
1660 (1)*	<i>pr pr pr V<sub>2</sub> v<sub>2</sub> v<sub>2</sub></i>	"	"	"

\* From table 4.



TABLE 4

*The genotypic constitution for the  $pr$  locus in non-virescent trisomes from colorless aleurone seeds.*

PEDIGREE	NUMBER OF PLANTS OF $Pr Pr pr$ CONSTITUTION	NUMBER OF PLANTS WITH $Pr pr pr$ CONSTITUTION	NUMBER OF PLANTS WITH $pr pr pr$ CONSTITUTION	TOTAL
1621 et cetera to 1663	62	106	1	169

shows that two of the  $v_2$  trisomes from colorless seed were also homozygous for  $pr$ . One hundred and sixty-nine non-virescent trisomes from colorless seed were either selfed or backcrossed by  $ACR pr$  plants. In either case a 1:1 ratio for colored:colorless aleurone resulted. One of these 169 plants was homozygous for  $pr$ . Therefore it is apparent that the doubling of the number of  $pr$  trisomes was justified in order to find the approximate number of  $pr$  trisomes in the total population.

The genotypic constitutions of the exceptional trisomic types listed in table 3 were determined by crossing with the appropriate testers. For example, the constitutions of the  $v_2$  trisomes with respect to the  $pr$  locus were determined by selfing and out-crossing with  $ACR pr$  individuals. Plant 1626-1 was a trisomic  $v_2$  plant from purple seed. That it had the constitution  $Pr v_2$  is shown by the following tests: (1) It was a trisome

$$\begin{array}{c} \underline{pr} \quad \underline{v_2} \\ \underline{pr} \quad \underline{v_2} \end{array}$$

by root tip counts and appearance. (2) When self-pollinated it gave 177  $Pr$ :95  $pr$  seeds which is close to a 2:1 ratio. (3) When used as the male parent on  $ACR pr$  silks it gave 62  $Pr$ :122  $pr$ , a good 1:2 ratio. (4) It was homozygous for  $v_2$  as shown by its appearance and when it was crossed with  $v_2$  plants gave only  $v_2$  individuals in the  $F_1$ . Another example is as follows: Plant 1630-2 was a trisomic  $v_2$  plant from purple seed. When selfed it gave 39  $Pr$ :17  $pr$  seeds and when used as the male parent in a back-cross gave 118  $Pr$ :234  $pr$  seeds. It was trisomic by appearance and root tip counts, and tests showed it to be  $v_2$ . These results are typical of those obtained for the other exceptional trisomes and they seem to make possible the statement that chromatid crossing over in *Zea* is a proven fact.

While the writer feels that a perfectly accurate classification into trisomes and disomes can be made by appearance alone, an attempt was made to secure root tip counts for all of the exceptional trisomic plants. These counts have been included in table 3.

Among the trisomic individuals listed in table 2 there were 4.1 percent of them homozygous for  $v_2$ . If the locus of  $v_2$  is far enough removed from the insertion region so that its distribution is at random with respect to the insertion region the percentage of trisomic plants homozygous for  $v_2$

should be 6.7. It would seem therefore that the locus of  $v_2$  was some distance (crossover distance—not physical distance) from the spindle fiber. If 18 be accepted as the approximate number of  $pr$  trisomes, this gives a percentage of 1.2. The locus of  $pr$  then should be much closer to the insertion region than is the locus of  $v_2$  since the frequency with which a gene becomes homozygous is a function of its crossover distance from the spindle fiber.

Five of the nine  $pr$  trisomes were also homozygous for  $v_2$  while four were non-virescent. If  $pr$  and  $v_2$  were closely linked and on the same side of the spindle fiber they would be expected to appear together in the exceptional trisomic types. But since the two loci are far apart (41 percent recombinations) there should be no tendency for the  $pr$  trisomes to be  $v_2$ , irrespective of whether or not they are in the same arm of the chromosome. One of the four green  $pr$  trisomes was of  $pr \quad V_2$  constitution. The egg was there-

$$\frac{pr \quad V_2}{pr \quad v_2}$$

fore  $pr \quad V_2$  in constitution. This suggests that  $pr$  and  $v_2$  are on opposite

$$\frac{pr \quad V_2}{pr \quad v_2}$$

sides of the insertion region since a much simpler type of prophase configuration will give the above combination if  $pr$  and  $v_2$  are in different arms than if they are in the same arm. This is in agreement with the genetic map of this chromosome which places the gene  $bm_1$  between  $pr$  and  $v_2$  and McCLINTOCK (1932) believes  $bm_1$  to be near the insertion. The cytogenetic data of the writer on a reciprocal translocation involving this chromosome also places  $pr$  much nearer the insertion than is  $v_2$ .<sup>2</sup>

#### THE ORIGIN OF A 32 CHROMOSOME PLANT

In the winter of 1931-32 a planting of seed of exactly the same cross as reported in this paper was made for the purpose of demonstrating the occurrence of chromatid crossing over. Since it was not considered possible to identify all of the trisomic plants in the seedling stage these data have not been included in table 2. They were, however, the basis for the statement by the writer in 1932 that crossing over occurred in *Zea* between chromatids. One of the  $v_2$  plants from a purple seed in these cultures was classified as a trisome. Root tip counts in several clear figures showed that this plant had 32 chromosomes. If the pollen contributed 10 of these chromosomes there is left a total of 22 chromosomes as the contribution from the egg. The plant was clearly  $v_2$  as progeny tests showed later,

<sup>2</sup> Data recently obtained from a study of this translocation give, in conjunction with McCLINTOCK's placing of the  $bm_1$  locus, the following order:  $bm_1$ —insertion region— $pr$ — $v_2$ .

so it is possible to say that an  $n+1$  megaspore, with two chromosomes carrying the recessive  $v_2$  gene as a result of chromatid crossing over, in some way doubled its number of chromosomes to 22. Whether this occurred through non-disjunction of the entire chromosome set during the formation of the embryo sac or through fusion of the egg nucleus with a synergid it is impossible to state. The fact remains, however, that a doubling of the chromosome number occurred during the gametophytic generation.

#### TRISOMIC RATIOS

If a trisomic plant of  $Pr\ Pr\ pr$  constitution is pollinated by recessive pollen a 5:1 ratio of  $Pr:pr$  is expected if 50 percent of the eggs are  $n+1$ . Since only 31 percent of the progeny from a plant trisomic for the  $pr-v_2$  chromosome are trisomes the theoretical ratio of  $Pr:pr$  is 3.35:1. If, however, we assume that crossing over occurs in a double strand stage and that the locus of the factor under observation is sufficiently far from the spindle fiber region so that it assorts at random with respect to the spindle fiber, the theoretical ratios are markedly changed. With 50 percent of trisomes and chromatid crossing over with randomness a 4:1 ratio of  $Pr:pr$  is expected instead of a 5:1 ratio with "chromosome" crossing over. Since only 31 percent of the plants are trisomes a ratio of approximately 3:1 (25.1 percent recessives) for  $Pr:pr$  is expected with chromatid crossing over and random assortment. It follows that the genetic ratio for a given factor pair in triploid individuals depends in part upon the location of the gene with respect to the point of spindle fiber attachment. That is, the further removed (crossover value) the locus of the genetic factor is, the greater the effect, up to the ratio expected with random assortment, will be upon the ratio of dominants to recessives. In other words if the locus of a factor is close to the insertion region its genetic ratio will be little affected by the occurrence of chromatid crossing over while a progressively greater disturbance in the genetic ratio will occur the further removed the gene lies from the insertion region. Thus the theoretical gametic ratio in duplex trisomic individuals with 31 percent of the progeny trisomes will be 3:1 with chromatid crossing over and random assortment while it will be 3.35:1 with "chromosome" crossing over. Therefore, the ratio of dominants to recessives in the progeny of triploid individuals should indicate whether the gene in question is close or far removed from the insertion region.

Since we have calculated the theoretical ratios expected on the various assumptions let us see what the observed ratios were for the two factor pairs reported in this paper. The percentage of  $pr$  seeds among a total progeny of 14,160 was 22.7 and the percentage of  $v_2$  plants in a total of 4856 individuals was 24.9. The expected percentage of recessives from a

duplex trisomic individual with 31 percent of its progeny trisomes and "chromosome" crossing over, or with chromatid crossing over and the location of the gene near the insertion, should be 23 (3.35:1 ratio). This suggests that *pr* should be close to the insertion region. With 31 percent of trisomes and chromatid crossing over with random assortment the expected percentage of recessives should be 25.1. There were 24.9 percent of *v*<sub>2</sub> plants which indicates that the locus of *v*<sub>2</sub> is far enough removed from the insertion so that an approach to a random assortment is realized. The observed percentages of *pr* and *v*<sub>2</sub> agree very well with the position of these loci with respect to the insertion region as determined from the frequency of homozygous trisomic types.

The difference between 25.1 percent and 23.0 percent, the two extremes, is small and a large amount of data would be necessary to permit any definite conclusions as to the locus of a gene. If 50 percent of the functioning eggs were  $n+1$  the difference in the percentage of recessives would be greater since 16.7 percent would be expected with chromosome crossing over as contrasted to 20 percent with chromatid crossing over and random assortment.

#### CROSSING OVER IN TRIPLOIDS

A theoretical discussion of the effect of double strand crossing over on crossover values in triploids and trisomics will be taken up in this section.

Represent the three homologous chromosomes present in a triploid or trisome as *a*, *b*, and *c* and let *c* carry the recessive genes. Crossing over can occur between *a* and *b*, *a* and *c*, and *b* and *c* with equal frequencies. Crossing over between *a* and *b* cannot be detected since both chromosomes carry the normal allelomorphs but crossing over between *a* and *c*, and *b* and *c* lead to detectable crossovers. Therefore, it can be argued that the amount of actual crossing over is  $3/2$  the observed amount (REDFIELD 1930). If there is a random distribution of the three chromosomes the proportion of crossover to non-crossover chromosomes should be the same in both the haploid and diploid eggs. REDFIELD (1930, 1932) apparently assumed this for she multiplied the observed crossover values determined from the diploid progeny for the factor  $3/2$ . If we make a similar calculation for the *pr-v*<sub>2</sub> region from the data in table 2 we find that the observed recombination value among the disomic offspring is 26.2 percent. The corrected value would be 39.3 percent which agrees very well with the recombination value of 40.6 percent found in the diploid controls. Since only the diploid offspring were used in the calculation, a similar recombination value, if crossing over is the same in mega- and microsporocytes, should be found if the trisomic plants were used as the male parent in a backcross with double recessive individuals. Here all the offspring can be used since none

of the  $n+1$  pollen succeeds in effecting fertilization. A corrected recombination value, using the factor  $3/2$ , of 41.0 percent was found.

In the discussion above it has been assumed that crossing over between  $a$  and  $c$ , and  $b$  and  $c$  lead to detectable crossovers. Let us examine the consequences of crossing over between  $b$  and  $c$ . For simplicity, we will assume that we are dealing with a rod-shaped chromosome and that the location of the two genes under observation is near the terminal insertion region. Represent the genes as  $x$  and  $y$  and the normal allelomorphs by the conventional  $+$  sign. The constitution of the triploid before crossing over takes place is:

$$\begin{array}{r} + \quad + \quad a \\ + \quad + \quad a' \\ + \quad + \quad b \\ + \quad + \quad b' \\ x \quad y \quad c \\ x \quad y \quad c' \end{array}$$

Crossing over occurs between chromosomes  $b$  and  $c$  but involves chromatids  $b'$  and  $c$ . The constitution following the crossing over is:

$$\begin{array}{r} + \quad + \quad a \\ + \quad + \quad a' \\ + \quad + \quad b \\ x \quad + \quad b' \\ + \quad y \quad c \\ x \quad y \quad c' \end{array}$$

If the distribution of the three chromosomes is at random in the metaphase of the first meiotic division (arbitrarily assumed to be reductional for the spindle region) there are six combinations possible at the end of the first division. They are as follows:

$$\begin{array}{llllll} (1) & (2) & (3) & (4) & (5) & (6) \\ \begin{array}{r} + \quad + \quad a \\ + \quad + \quad a' \end{array} & & \begin{array}{r} + \quad + \quad a \\ + \quad + \quad a' \end{array} & & \begin{array}{r} + \quad + \quad b \\ x \quad + \quad b' \end{array} & \\ \begin{array}{r} + \quad + \quad b \\ x \quad + \quad b' \end{array} & \begin{array}{r} + \quad y \quad c \\ x \quad y \quad c' \end{array} & \begin{array}{r} + \quad y \quad c \\ x \quad y \quad c' \end{array} & \begin{array}{r} + \quad + \quad b \\ x \quad + \quad b' \end{array} & \begin{array}{r} + \quad y \quad c \\ x \quad y \quad c' \end{array} & \begin{array}{r} + \quad + \quad a \\ + \quad + \quad a' \end{array} \end{array}$$

The next division is equational for the spindle region so 12 of the resulting 24 combinations have 2 chromosomes represented and the other 12 have a single chromosome. But 4 of the 12 single chromosome types are crossover chromosomes and 8 are non-crossovers and 8 of the 24 chromosomes in the "two chromosome" combinations are crossover strands. That is, instead of every crossover between  $b$  and  $c$  leading only to detectable cross-

over chromosomes we find that only one-third of the recovered chromosomes are crossovers whereas in diploids one-half of the recovered strands, from every crossover point, are crossovers. Therefore, in place of using the factor  $3/2$  to obtain the actual amount of crossing over it is necessary to use the factor 2.25.

If the factor 2.25 is the correct one to use and the factor  $3/2$  used by REDFIELD is wrong, then her comparisons between triploid and diploid crossing over must be reevaluated since her triploid values were obtained by multiplying the observed crossover values by  $3/2$ .

In a comparison of crossing over in triploids and diploids it is necessary to state the basis upon which the comparison is to be made. In diploids the frequency of crossover points in a short region is always twice the map distance since only one-half of the strands exchange segments at the crossover point. Therefore, instead of saying that genes A and B are 10 map units apart it would be equally proper to say that 20 cells out of every 100 have a crossover point between the loci A and B. We can then express map distances in terms of the number of crossover points as well as the percentage of crossover chromosomes among the progeny. In diploids a map distance of 10 means a crossover point frequency of 20 percent. If we wish to compare crossing over in a certain region in triploids with that in diploids we must keep in mind that what we really want to measure is the frequency with which crossover points occur in that region. We know that in triploids only two of the three chromosomes are synapsed at a given level, with the third chromosome acting as an univalent (cytological observations on plant sporocytes), while the *Drosophila* genetic data show that crossing over occurs between only two of the three chromosomes at any level. Therefore, in a short region we can have only one crossover point in triploids and only one in diploids. If we wish to compare the total amount of crossing over for a given region in triploids with that in diploids we must bear in mind that what we have to measure is the frequency with which crossover points actually occur in that region rather than the frequency with which crossover strands are found in the progeny. Since the proportion of crossover chromosomes recovered from a crossover point in triploids is not the same as in diploids, as shown on page 546, this difference must be taken into account. The writer believes that the proper way to "compare the actual amount of crossing over in triploids with that in diploids" is to compare the actual frequency of crossover points in the two forms rather than the resulting frequencies of types observed in the progeny. In other words, if we are to reach a real understanding of crossing over, we should study the mechanism involved and not confine our attention to the results of its action. It is upon these grounds that it is suggested that REDFIELD failed to use the proper correction factor, since

she explicitly stated that she was making the corrections to obtain the actual amount of crossing over in triploids.

All of the foregoing calculations have been based on the assumption that there was a random distribution of the three chromosomes in the first meiotic division. What would be the consequences if there should be a correlation, either positive or negative, between crossing over and disjunction? The data presented in this paper do not aid in solving this question but the *Drosophila* triploid data can be utilized. BRIDGES and ANDERSON (1925) studied crossing over in the X chromosome of triploid *Drosophila*. The three X chromosomes carried mutant genes so situated that the identity of a considerable portion of any of the recovered strands could be established. Approximately 41 percent of the progeny from a triploid mother are diploid females whose two X chromosomes come from their mother. These females are XXY in constitution and are called exceptional daughters since both of their X chromosomes came from their mother. These exceptional daughters were mated and the constitution of their X chromosomes determined from their male offspring. The analysis of the genotypic constitution of the exceptional daughters permitted a calculation of the amount of crossing over which occurred in the triploid mothers. These crossover values were then compared with the values found for the same regions in the diploid controls.

*Comparison between triploid and diploid crossing over for the X chromosome  
(after BRIDGES and ANDERSON 1925)*

REGION	TRIPLOID CROSSOVER VALUE	DIPLOID CROSSOVER VALUE	RATIO $\frac{T}{D}$
1	14.3	6.9	2.07:1
2	11.3	22.8	0.50:1
3	3.9	10.1	0.39:1
4	8.2	16.2	0.51:1

For the rightmost regions (nearest the spindle fiber) the ratio of triploid to diploid crossing over was about 1:2, while for the leftmost region the ratio was approximately 2:1. These ratios point to a real difference in the amount of crossing over in triploid and diploid females. But it is important to remember that the triploid values were calculated from those eggs which had received two maternal X chromosomes.

REDFIELD (1930, 1932) studied crossing over in the II and III chromosomes in triploid females. The amount of crossing over in the triploids was based on the constitution of those eggs which received a single chromosome from the mother. The calculated amounts of crossing over, using the correction factor  $3/2$ , in the triploid for the various regions studied are compared below with the crossover values for the diploid control females.

*Comparison of triploid and diploid crossover values for the II chromosome (after REDFIELD 1932)*

REGION	TRIPLOID CROSSOVER VALUE	DIPLOID CROSSOVER VALUE	QUOTIENT $\frac{T}{D}$
<i>al-dp</i>	8.3	10.0	0.83
<i>dp-b</i>	16.2	27.2	0.59
<i>b-pr</i>	7.4	5.7	1.30
<i>pr-c</i>	27.1	19.2	1.41
<i>c-px</i>	13.0	22.1	0.59
<i>px-sp</i>	4.4	5.7	0.77

*Comparison of triploid and diploid crossover values for the III chromosome (after REDFIELD 1930)*

REGION	TRIPLOID CROSSOVER VALUE	DIPLOID CROSSOVER VALUE	QUOTIENT $\frac{T}{D}$
<i>ru-h</i>	19.5	25.3	0.77
<i>h-th</i>	14.9	15.3	0.97
<i>th-st</i>	1.2	0.4	3.00
<i>st-cu</i>	21.2	5.6	3.79
<i>cu-sr</i>	14.6	14.0	1.04
<i>sr-es</i>	6.1	8.9	0.69
<i>es-cu</i>	18.0	34.3	0.52

The location of the spindle fiber in the II chromosome is slightly to the right of *pr* while the spindle fiber is situated between *st* and *cu* in the III chromosome. Therefore, we see that in REDFIELD's experiments where her calculations of triploid crossing over are based on the constitution of those eggs which received a single chromosome the calculated amount of crossing over near the insertion region in triploids is higher than for the corresponding regions in diploids. The relative amount of crossing over in triploids in the distal regions of the II and III chromosomes is less than in the diploid controls. These results are the converse of those found by BRIDGES and ANDERSON. Since REDFIELD's calculations were based on those eggs which received a single strand while BRIDGES and ANDERSON's were obtained from those eggs which received two strands it seemed plausible to the writer that there might be a direct relationship between crossing over in triploids and the distribution of the members of the trivalent group. Especially did this seem likely since ANDERSON's (1929) data showed that non-disjunction of the two X chromosomes in diploid females was more likely to happen if there was little or no crossing over between the two chromosomes. To tell if there is a correlation between crossing over and disjunction in triploids it is best to study both those eggs which receive a single strand and those which receive two strands. Unfortunately the published data do not permit such a direct comparison but the data of BRIDGES and ANDERSON on the types of association in the two chromosome combinations do permit some tentative conclusions to be drawn.

We will for the present confine our interest to the rightmost regions



(nearest the spindle fiber) studied by BRIDGES and ANDERSON. Assume that a crossover occurs between chromosomes a and b near the insertion region. We know that only one of the chromatids from chromosome a and only one chromatid from chromosome b are involved in the crossover. The two chromatids from chromosome c are not involved in any crossover in this region (BRIDGES and ANDERSON). The identity of the six strands following the crossing over is as follows: a    a

b	a'
a'	b
b'	b'
c	c
c'	c'

If we assume that crossing over near the insertion region has no effect on the distribution of the chromosomes, then six types of combinations are possible at the end of the first meiotic division (we are arbitrarily assuming that reduction occurs at the first division since it is immaterial whether the first or second division is reductional for the insertion region). After the second (equational) division 24 combinations are expected with equal frequencies. The "two chromosome" combinations containing crossover strands can be classified into the following types of association:

<i>Types of association</i>	<i>Frequency</i>	
<u>crossover</u>	b    a	4
dissimilar non-crossover	c    c	
<u>crossover</u>	b    a	2
similar non-crossover	b    b	
<u>complementary crossover</u>	b    a	1
complementary crossover	a    b	

The single chromosome combinations are composed of 4 crossover to 8 non-crossover strands and the percentage of crossover strands is 33.3 among the single strands. There are twelve "two chromosome" combinations which comprise a total of 24 strands. Eight of these are crossover chromosomes so the percentage of crossover strands is here also 33.3. Therefore if the distribution of the three chromosomes (six chromatids) is not influenced by crossing over near the insertion region the same amount of crossing over should be found in both types of eggs.

But assume that crossing over near the insertion region does have an effect on the distribution of the chromosomes and, since ANDERSON's 1929 data gives such an indication, further assume that if two chromosomes undergo crossing over near their insertion regions they always pass to

different poles. As before the identity of the six strands following the postulated crossover is:

a	a
b	a'
a'	b
b'	b'
c	c
c'	c'

But since the mode of disjunction has been determined by the previously occurring crossover we have only four instead of six combinations at the end of the first division. And instead of 24 combinations only 16 are expected at the end of the second division. The "two chromosome" combinations containing crossover strands can be resolved into the three types of association:

	<i>Frequency</i>	
<u>crossover</u>	<u>b</u> <u>a</u>	4
dissimilar non-crossover	c c	
<u>crossover</u>	<u>b</u> <u>a</u>	0
similar non-crossover	b b	
<u>complementary crossover</u>	<u>b</u> <u>a</u>	0
ccmplementary crossover	a b	

There are 16 strands in the "two chromosome" combinations and 4 of these are crossover chromosomes which is a percentage of 25.0. Among the single strand combinations the number of crossover strands is 4 out of a total of 8 or a percentage of 50.0. Obviously if there is a correlation between crossing over near the insertion and distribution, it makes a great difference in the observed crossover values which class of eggs are studied. With complete correlation and using those eggs which received two chromosomes the amount of crossing over would be only one-half that which would be found if the eggs with only one chromosome were used. BRIDGES and ANDERSON found the amount of crossing over in the rightmost region in the triploid mother was only one-half that in the diploid. Their calculations were based on those eggs which received two chromosomes. REDFIELD, working with eggs which received a single chromosome, found the amount of crossing over in the regions near the spindle fiber to be from one and one-half to more than three times as much as in the diploid controls. It would seem that such differences might have some relation to crossing over and the manner of disjunction.

In table 12 of the paper by BRIDGES and ANDERSON (1925) are listed the various associations of crossover chromosomes from triploid females. As stated before if there is no correlation between crossing over

and disjunction there should be for regions near the insertion region a ratio of  $4 \frac{b a}{c c} : 2 \frac{b a}{b b} : 1 \frac{b a}{a b}$  combinations. With complete correlation the ratio should be  $4 \frac{b a}{c c} : 0 \frac{b a}{b b} : 0 \frac{b a}{a b}$ . The data of BRIDGES and ANDERSON for their rightmost region, the right end of which is approximately ten units from the fiber, show  $23 \frac{b a}{c c} : 1 \frac{b a}{b b} : 3 \frac{b a}{a b}$  combinations. This is far from the 4:2:1 ratio expected with random disjunction and strongly supports the idea that there is a positive correlation in triploids between crossing over near the fiber and disjunction to opposite poles. In fact the correlation may be complete for regions very near the fiber as the deviation from the 4:0:0 ratio for the rightmost region may well be due to the fact that it was some 10 units from the end. Their data also show that for the leftmost region the approximation to a  $4 \frac{b a}{c c} : 2 \frac{b a}{b b}$  ratio is very close, which suggests that the effect of crossing over on disjunction is dissipated progressively away from the fiber. This would be expected.

If there exists a positive correlation between crossing over and disjunction the association of strands in the diploid eggs would be such that the percentage of exceptional daughters homozygous for a recessive gene whose locus is near the fiber end would be low, approaching zero as a limit. But since the effect of crossing over on disjunction becomes less away from the spindle fiber attachment point the percentages of exceptions homozygous for genes in the distal end should increase progressively. Only 1.1 percent of the exceptional daughters in BRIDGES' and ANDERSON's data were homozygous for point V (*f*, *B*, +), approximately 10 units from the fiber, while 11.5 percent of them were homozygous for point I (*y*, *sc*, +), which is about 70 units from the fiber attachment point. RHOADES (1931) in a study of homozygosis in diploid females with attached X's, where crossing over cannot affect disjunction since that is predetermined, found 19.0 percent of the exceptional daughters homozygous for yellow (*y*) and about 5 percent of homozygosis for forked (*f*). The ratio of homozygosis for *y* and *f* in the attached X data is 3.8:1 while it is 10.4:1 in the triploid data. It is possible that this difference is due, in part at least, to the correlation between crossing over and disjunction in triploids. The marked differences in coincidence values in triploids and diploids found by BRIDGES and ANDERSON should also affect the frequencies of homozygosis.

Although the relation of crossing over to disjunction is admittedly more complex in V shaped chromosomes than in rod shaped it seems to the writer that the regional differences in crossing over between triploids and

diploids reported by REDFIELD and by BRIDGES and ANDERSON cannot be accepted until the possible relationship between crossing over and disjunction has been considered, and if the writer is correct in his argument, until the proper factor for undetected crossovers has been used. The correction factor for the distal regions in REDFIELD's data should then be 2.25 instead of 1.5. We must assume, however, that there is for these regions no effect of crossing over on disjunction. For those regions near the insertion and where a strong correlation between crossing over and disjunction presumably exists the correction factor is 1.5. The difference in the correction factors for the distal and proximal regions is due to the correlation between crossing over and disjunction which results in a higher percent of crossovers in the proximal regions going to the haploid eggs. The correction factors for intermediate regions lies somewhere between these two values.

The above correction factors are to be used when only one of the three chromosomes is marked by mutant genes. If all three of the chromosomes are properly populated with mutant genes so that crossing over can be detected between all of the three homologues, different correction factors must be used. The correction factor for regions near the insertion becomes 1.0 while for distal regions, where no correlation exists between crossing over and disjunction, the correction factor should be 1.5. These correction factors are to be used when those eggs which receive a single chromosome are studied. If, as BRIDGES and ANDERSON did, those eggs which receive two chromosomes are studied, and all three of the chromosomes are marked by mutant genes, the correction factor for regions near the insertion should be 2.0 and for the distal regions it should be 1.5. If these correction factors are applied to BRIDGES' and ANDERSON's data the amount of crossing over near the insertion is approximately the same in triploids as in diploids, while the amount of crossing over in the leftmost region of the X chromosome becomes even greater in triploids than in diploids.

The corrected percent of recombination of 39.3 for the  $pr-v_2$  region was determined from the diploid offspring listed in table 2 of this paper but the correction factor used in obtaining this value was 1.5. As pointed out in a preceding section it is in close agreement with the percent of recombination found in the disomic controls and would seem to permit the conclusion that the amount of recombination in this region was approximately the same in trisomes and disomes. But if the factor 1.5 is not the one to use, as the writer argues, the corrected value of 39.3 is incorrect and a recalculation must be made using the proper correction factor. Since  $v_2$  is some distance from the insertion region the correction factor should probably be much nearer 2.25 than 1.5.

## ADDENDUM

Recently MATHER (1933) attempted to calculate the frequency of chiasmata in triploid *Drosophila*. He used crossover values from REDFIELD's data which were based upon the constitution of the diploid offspring. His calculations led him to conclude that there was an excess of crossover chromosomes among the diploid progeny. To account for this calculated excess of crossovers he postulates that in triploids the three homologous chromosomes are associated as a trivalent group in two-thirds of the cases and in the remaining one-third as a bivalent and an univalent. Since crossing over can only occur between the members of the bivalent, and the univalent will pass at random to either pole, this would lead to an excess of crossovers among the diploid progeny.

The writer agrees with MATHER that there is an excess of crossovers among the diploid progeny. He does not, however, entirely agree with MATHER's explanation of their occurrence although it is both possible and probable that some of the excess crossovers are caused by the formation of some bivalents and univalents instead of trivalent groups. But before accepting MATHER's explanation the following facts should be mentioned:

(1) The excess of crossovers among the diploid offspring could be at least partially accounted for by the correlation between crossing over near the insertion and disjunction.

(2) The data of BRIDGES and ANDERSON (1925) and REDFIELD (1932) show that the two types of double crossovers (recurrent and progressive) occur with approximately equal frequency. This suggests that the frequency with which the three homologous chromosomes have failed to synapse so as to form a trivalent group at metaphase I is low (assuming the occurrence of a univalent is due to its failure to pair with the other two homologues in the meiotic prophase).

(3) MATHER's assumption of univalents being formed in  $33\frac{1}{3}$  percent of the cases is not universally valid since the frequency of trivalents at metaphase I in maize plants trisomic for the *pr-v*<sub>1</sub> chromosome is approximately 90 percent.

## SUMMARY

1. The occurrence of chromatid or double strand crossing over in *Zea* was genetically demonstrated by the determination of the genotypic constitution of certain trisomic types.

2. The frequencies of homozygosis for *pr* and *v*<sub>1</sub> indicate that *v*<sub>1</sub> is much further removed from the insertion region than is *pr*.

3. The effect of chromatid crossing over upon genetic ratios in triploids and trisomes is discussed.

4. Genetic proof was obtained for the occurrence of the doubling of the entire chromosome set in the gametophytic generation.

5. The genetic data obtained by BRIDGES and ANDERSON, and REDFIELD for triploid *Drosophila* is discussed with reference to a possible correlation between crossing over in triploids and disjunction. Certain data of BRIDGES and ANDERSON are presented in support of a positive correlation between crossing over and disjunction.

6. REDFIELD's treatment of her triploid data is discussed and the suggestion is made that she failed to use the proper correction factor for undetected crossing over. It is further suggested that the triploid data of BRIDGES and ANDERSON should also be corrected.

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